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ONCOLOGY OVERVIEW

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ORIGINAL

SELECTED ABSTRACTS

ON

AFLATOXIN AND OTHER MYCOTOXIN CARCINOGENESIS

SEPTEMBER 5, 1978

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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National Cancer Institute

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INTRODUCTION

This OVERVIEW on mycotoxin carcinogenesis is a compilation of abstracts covering publication years 1972 to 1977 that were selected from the CANCERLIT computerized literature data base. It covers all aspects of study on mycotoxins, including epidemiology, formation and occurrence, toxicity, metabolism, tumor induction, cellular effects, and general reviews.

The strategy used to obtain these abstracts is available on request to the CIDAC and can be used to update this OVERVIEW by any MEDLARs (MEDLINE Terminal System) (see back cover).

This OVERVIEW was prepared by Arthur Furst (editor), and William E. Davis, Jr., Barbara L. Thompson, and John S. Krebs of the staff of the CIDAC for Carcinogenesis Information, SRI International, Menlo Park, California.

EDITORIAL COMMENTARY

In the past 20 years, a number of very potent carcinogens of natural origin have been found and the possibility that certain of them may be factors in human cancers—in particular, liver cancer—has been extensively investigated and discussed. This group includes aflatoxins and other mycotoxins as well as several normal plant constituents.

The evidence for relating aflatoxin consumption and human liver cancer has been summed up by Shank (see supplementary list for references): 1) Aflatoxin B1 is the most potent animal hepatocarcinogen yet found; 2) Although species' sensitivity varies, all animal species tested with aflatoxin B1 developed liver tumors; 3) The human liver contains enzymes capable of metabolizing aflatoxin B1, although the proximate carcinogen for aflatoxin B1 has not yet been established; 4) Geographical areas where foodstuffs are extensively contaminated by aflatoxins correspond to areas with increased incidences of human liver tumors, and in three instances, a close relationship has been found between aflatoxin consumption and primary liver cancer; 5) In addition, the Reye-Johnson syndrome, a children's disease circumstantially associated with aflatoxin consumption, parallels the geographical distribution of primary liver cancer.

Although aflatoxin B1 is the most potent carcinogen of the aflatoxins, most of the others such as G1, M1, and B2 also show carcinogenic activity.

Other mycotoxins such as sterigmatocystin, cyclochlorotine, griseofulvin, and luteoskyrin have been found to be carcinogenic in animal studies, but are not nearly as potent as aflatoxin B1.

The metabolism of the aflatoxins, particularly B1, is crucial to the eventual toxicity since microsomal enzyme activation is required prior to cytotoxicity. Thus, an understanding of the complex metabolic pathways of the aflatoxins may help elucidate the toxicological significance of these carcinogenic compounds. With this in mind, many of the abstracts in this OVERVIEW deal with the metabolism of the aflatoxins rather than their direct carcinogenicity.

It should be noted that aflatoxin B1 is a transplacental carcinogen. This phenomenon should be considered in future studies.

SUPPLEMENTARY REFERENCES ON MYCOTOXINS

1. R. C. Shank. Epidemiology of aflatoxin carcinogenesis. Adv. Mod. Toxicol. 3, 291-318 (1977).

2. M. Enomoto et al. Carcinogens produced by fungi. Ann. Rev. Microbiol. 26, 279-312 (1972) (133 ref).

3. P. M. Newberne. Mycotoxins: Toxicity, carcinogenicity and the influence of various nutritional conditions. Environ. Hlth. Perspect. 9, 1-32 (1974).

4. P. K. Austwick. Mycotoxins. Brit. Med. Bull. 31(3), 222-229 (1975).

5. G. W. Wogan. Mycotoxins and other naturally occurring carcinogens. Adv. Mod. Toxicol. 3, 263-290 (1977).

6. Proceedings of the First International Conference on Mycotoxins in Human and Animal Health. J. V. Rodricks, E. W. Hesseltine, and M. A. Mehlman (eds). Pathotox Publishers, Inc., Park Forest South, Illinois (1977).

I. THE ROLE OF MYCOTOXINS IN HUMAN CANCER AND IN OTHER DISEASES

A. Association of Mycotoxins, Primarily Aflatoxin, with Human Neoplasms

1. THE ASSOCIATION OF NEOPLASTIC DISEASES AND MYCOTOXINS IN THE ENVIRONMENT.

Aleksandrowicz J, Smyk B
Med. Acad., Cracow, Poland
Tex Rep Biol Med; 31(4):715-726 1973

The fungal flora were examined in 210 human dwellings of patients with clinical tumor disease and 170 dwellings of clinically healthy persons. Penicillium meleagrinum was the species most often encountered in the dwellings of patients, followed by Aspergillus flavus, Cladosporium herbarum, Rhizopus nigricans and Alternaria geophila. The most frequently found species in homes of patients with tumors were Penicillium meleagrinum and, slightly less often, Aspergillus flavus. Mycoflora in dwellings of married couples in which both partners suffered from tumors simultaneously or at intervals of 1-2 yr, showed Aspergillus flavus in 8 of 9 dwellings, compared with 2 of 9 control dwellings. Penicillium meleagrinum and Cladosporium herbarum were also relatively frequent in this group. In homes of cancer patients, Aspergillus flavus and Penicillium meleagrinum predominated. In dwellings of leukemia patients, Penicillium meleagrinum was most frequent, followed by Aspergillus flavus and Cladosporium herbarum and Rhizopus nigricans. These results indicate the presence of a higher incidence of fungi-producing oncogenic mycotoxins in the home environment of tumor patients.

2. SOME ILLUSTRATIVE SYSTEMS OF CHEMICAL CARCINOGENESIS: (4) AFLATOXIN AND OTHER NATURALLY OCCURRING CARCINOGENS. (PP. 302-309)

Warwick GP
Scientific Foundations of Oncology. Symington T, Carter RL, ed. London, William Heinemann Medical Books Ltd, 1976.

The author discusses the toxic and carcinogenic properties of the mycotoxin, aflatoxin, its metabolism in experimental systems, and its possible involvement in the etiology of liver cancer in man. Epidemiological investigations in Kenya and the Far East have indicated that aflatoxin contamination of foods may be an important factor in the etiology of primary hepatocellular carcinoma. It is, however, important not to underestimate contributions from other possible predisposing factors. Poor nutritional status, preexisting liver disease, and other chemicals and viruses could act synergistically with the mycotoxin. Indeed, the absence of significant cirrhosis produced by aflatoxin B1 in most animals and the observation that most primary hepatocellular carcinomas in man arise from a cirrhotic liver could be reconciled by invoking the possibility of synergism with some such factor. Also, most primary hepatocellular carcinomas in man produce a-fetoprotein, whereas tumors induced in experimental animals by aflatoxin appear not to do so. Brief discussion is also made of the occurrence and properties of the pyrrolizidine alkaloids, cycasin, nitrosamines, and nitrosamides and of the possible involvement of other mycotoxins in the etiology of human liver cancer. (41 refs)

3. MYCOTOXICOSIS IN EUROPE IN THE 20TH CENTURY.

Berglund F
Medical Res. Dept., AB Kabi, S-104 25 Stockholm, Sweden
Lakartidningen; 72(51):5065-5066 1975

General toxicological data on mycotoxins and some statistical data on mycotoxicoses in Europe are presented. Aflatoxins have been found to be carcinogenic, and they can induce tumors in the liver, stomach, kidney, salivary glands, and intestine. Aflatoxin B1 appears to be most potent of all these compounds. In Sweden, they are present in high concentrations in peanut oil cakes and in lower concentrations in peanut butter and Brazil nuts. The max allowable aflatoxin concentration in Sweden is 600 ug/kg for fodder and 5 ug/kg for food for human consumption. (5 refs)

4. AFLATOXINS AND HEPATIC CANCER.

Payet M
No Affiliation Given
Med Chir Dig; 2:57 1975

There is some controversy about the role of aflatoxins in the pathogeny of liver cancers. The fungus Aspergillus flavus may contaminate not only the peanut but all oil-yielding plants. Industrial extraction processes guarantee the elimination of all traces of aflatoxin from cooking oils, so the role of aflatoxins can only be considered in relation to the human consumption of the residues from these processes (oil cakes). Although this consumption would seem to be equal for both sexes, primary cancer of the liver occurs five times more frequently in men than in women. In 1963, experiments showed that following the addition of a weak dose of aflatoxin to their diet, white Wistar rats developed cancer within 3 mo, after a phase of cirrhosis. These results have been achieved experimentally only in selected purebred rats. Wild rats, even in countries with high peanut and oil cake contamination, do not spontaneously develop cancer. Peanut contamination exists in all countries of the tropical zone and yet, only some of these countries have a high rate of hepatic cancer. Aflatoxins are probably not an exclusive or specific factor but just one possible factor among others in the genesis of this cancer. In conclusion, one "very curious" incident is described. During an experiment in Dakar, Senegal, in 1952, several infants of 6 to 12 mo received peanut oil cake food supplements. At the age of 7, two of these children presented definite histological alterations of the liver. A biopsy of one of them revealed a precirrhotic condition. In 1970, it was learned that one had died from a confirmed hepatic carcinoma. Reference samples of the original oil cakes were traced and found to be contaminated by Aspergillus flavus.

5. SOME CHARACTERISTICS OF MOZAMBICAN SHANGAANS WITH PRIMARY HEPATOCELLULAR CANCER.

Kew MC, Marcus R, Geddes EW
Dept. Medicine, Johannesburg Hosp., Johannesburg, South Africa
S Afr Med J; 51:306-309 1977

Shangaans from Mozambique suffer the highest incidence of primary hepatocellular cancer (PHC) in the world: the incidence in men is 98.2/100,000, accounting for 65.5% of all cancers. Certain characteristics of 328 male Shangaans with PHC were compared with those of 163 male Shangaans with hepatomegaly from causes other than PHC and with those of 122 non-Shangaan blacks with PHC. The mean age of the Shangaan PHC patients was significantly less (33.4 yr) than that of the non-Shangaans (40 yr). Alpha-fetoprotein was detected in 71.4% of the Shangaan PHC group and 61% of the non-Shangaan group. Cirrhosis of the nontumorous part of the liver was seen at necropsy in 62% of the Shangaans and in 66% of the non-Shangaans. Hepatitis B virus surface antigen was detected in the sera of 60% of the Shangaans with PHC, 53.4% of the non-Shangaans, and 9% of the Shangaans without PHC. Discussion is made of the possible etiological role of hepatitis B virus in cirrhosis and PHC. The limited data regarding the dietary history of each of the groups did not point to any outstanding differences. All subjects ate groundnuts and cycad pips from an early age, and they could have been exposed to mycotoxins and cycasin. (22 Refs)

6. PRIMARY CARCINOMA OF THE LIVER.

Anthony PP
Bland-Sutton Inst. Pathology, Middlesex Hosp. Medical Sch., London, England
Ann R Coll Surg Engl; 58(4):285-292 1976

Primary carcinoma of the liver is discussed. Although the carcinoma is rare in Western countries, it is one of the most common forms of malignancy in most parts of sub-Saharan Africa and the Far East. Liver-cell carcinoma affects younger people and shows a strong male predominance; bile duct carcinoma is a tumor of older people and is seen in both sexes. The histology of the tumors is presented. The clinical presentation, laboratory findings, paramalignant syndromes, alpha-fetoprotein test, and etiology are discussed. Although the association of malignancy and cirrhosis is recognized throughout the world, the mechanism of malignant transformation is not understood. **The role of mycotoxins,** hepatitis B antigen and commonly-used steroid drugs are considered. (27 refs)

7. AFLATOXINS AND THEIR RELATION TO HEPATOCELLULAR CARCINOMA. (PP. 25-41)

Wogan GN
Massachusetts Institute of Technology, Cambridge, MA
Hepatocellular Carcinoma. Okuda K, Peters RL, ed. New York, John Wiley and Sons Inc, 1976.

Chemically, aflatoxins are highly substituted coumarins that contain a fused dihydrofurofuran configuration peculiar to a limited number of compounds of natural origin. Aflatoxins have carcinogenic activity in many species of animals, including rodents, nonhuman primates, birds, and fish. The liver, in which the toxins induce hepatocellular carcinomas and other tumors, is the organ principally affected. Considerable research is being done on the metabolism of aflatoxin B1 to determine its mode of action and the differences in susceptibility among various animal species. The epoxidation pathway may represent a significant activation step in aflatoxin metabolism. Aflatoxins provide structural homologs for investigations of relationships between chemical structure and biological activity. They are among the few chemically identified and widely disseminated environmental carcinogens for which quantitative estimates of human exposure have been sought systematically. The risk of exposure is evidently much less in technologically developed countries than in developing areas. Although the absolute values of aflatoxin ingestion appear small quantitatively, their potency as carcinogens in animals must be kept in mind in order to put data into perspective. (58 Refs)

8. AFLATOXINS AND CANCER OF THE LIVER (MEETING ABSTRACT).

Wogan GN
Massachusetts Institute of Technology, Cambridge, Massachusetts
Third International Symposium On Detection And Prevention Of Cancer; 119 1976

Aflatoxins are among the few chemically defined and widely disseminated environmental carcinogens for which quantitative estimates of human exposure have been systematically collated. Studies have been carried out in several regions of Africa and Asia designed to determine aflatoxin exposure in population groups known to experience widely differing incidence of liver cell carcinoma. Incidence patterns of liver cancer in Swaziland and Uganda were compared with frequency of occurrence of aflatoxins in food staples. Diets of tribal groups with elevated cancer incidence were contaminated with aflatoxins at high frequency. In other studies in Thailand, Kenya, and Mozambique, aflatoxin ingestion has been quantitatively estimated in subpopulations in which liver cancer incidence varied over a wide range. In every instance, elevated cancer incidence was associated with highest levels of aflatoxin intake. In view of the potency of aflatoxins as liver carcinogens in animals, these data collectively suggest that aflatoxins are also carcinogenic to man and that regular ingestion of foods heavily contaminated with them increases the risk of liver cancer. (Author Abstract)

9. PRESENCE OF AFLATOXIN B1 IN HUMAN LIVER IN THE UNITED STATES.

Phillips DL, Yourtee DM, Searles S
Dept. Biochemistry, Cancer Res. Center, Business Loop 70 and Garth Ave., Columbia, MO 65201
Toxicol Appl Pharmacol; 36(2):403-406 1976

A sample of liver tissue was obtained at biopsy from a 56-yr-old man with carcinoma of the rectum and liver to study the in vitro metabolism of aflatoxin B1 . The toxin, apparently environmentally ingested, was present in amounts up to 520 ng/g of wet liver. No other metabolites were detected. Upon incubation of a portion of the liver homogenate with an NADPH-generating system, the amount of aflatoxin B1 in the liver chloroform-extractable fraction was reduced to 250 ng/g, and another metabolite of higher polarity was observed, to the extent of 40 ng/g. Re-evaluation of the scope and hazard from aflatoxin contamination in the US is suggested. (11 refs)

10. TROPICAL CIRRHOSIS AND HEPATOMA.

Coady A
Medical Res. Council Labs., Fajara, The Gambia, West Africa
J R Coll Physicians Lond; 10(2):133-114 1976

The controversial etiology (nutritional vs infectious) of cirrhosis and primary hepatoma in Third World countries is reviewed. The evidence for a toxic etiology is largely indirect. **Aflatoxin** (AF) can in animal models lead to hepatoma after varying intervals without any discernable chronic process. AF has an acute DNA-blocking effect and has been shown to interact with another group of naturally occurring hepatotoxins, the pyrrolizidine alkaloids (PA). Both AF and PA may affect immunocytes in veno-occlusive disease, and hepatocytes and immunocytes may be deleteriously exposed to similar substances simultaneously with hepatocyte colonization by a virus that the organism cannot eliminate without a fully active immune system. The immune response to the virus might be impaired concurrently with direct chemical toxicity to the liver cells. An aspect of HB infection of possible relevance is the increase in antigen titer that precedes clinically patent acute disease, coincident with a wave of DNA polymerase activity. Since part of the structure of HB antigen is host derived, it has been proposed that genetically determined nonrecognition of HG antigen may select for survival in undeveloped societies. The incidence of lymphomas has been associated with toxigenic fungi in Eastern Europe. Hepatoma may result from a runaway mutant arising in a regenerating hepatocyte in cirrhosis. In a noncirrhotic liver, a cell leached free of DNA-arresting AF or PA might resume uninhibited replication with a consequently damaged template mechanism. Unrestrained liver cell activity can give rise to a-fetoprotein that might suppress anticancer immunosurveillance. Protozoal or schistosomal suppression of the defensive response to viral infection could be a factor, along with other bacterial factors. The diseased liver may have a grossly impaired ability to handle chemical compounds. Regarding the nutritional theory of the origin of these disorders, the cation environment may be of importance. The impressive prevalence of HB antigenemia among people with and without disease continues to raise the question of the primary etiologic agent.

11. PRIMARY LIVER CANCER, ALPHA1 FETOPROTEIN AND HEPATITIS B ANTIGEN IN PAPUA, NEW GUINEA.

Woodfield DG, Endo Y, Matsuhashi T
Red Cross Blood Transfusion Service, Papua, New Guinea
Aust NZ J Med; 4(1):3-7 1974

Sera from 72 Papua, New Guinean patients with hepatocellular carcinoma were tested for alpha1 fetoprotein (AFP), hepatitis B antigen (HBAg) and antibody by sensitive techniques including radioimmunoassay. AFP was detected in 98% of samples and HBAg in 82%. The uniformity of immunological results might be related to a previously noted histological uniformity of the hepatic tumor types found in Papua, New Guinea. It also suggests that a single etiological factor may be causing liver cancer in Papua, New Guinea, possibly naturally occurring hepatotoxins and fungal contaminants of food, mainly aflatoxins. Hepatitis B antibody was present in only 2.8% of samples, suggesting an immune defect in liver cell cancer patients. The low level of HB antibody in patients with no detectable HBAg may indicate an immune reaction absorbing any antibody produced, and removing antigen from the sera.

12. HEPATITIS AND HEPATOMA IN TAIWAN.

Sung JL
Jpn J Gastroenterol; 70:977-985 1973

Viral hepatitis is a common disease in medical practice in Taiwan. From a survey study for university students, the incidence of anicteric hepatitis was estimated to be more than 2%. The food contaminated with this virus might be the source of infection. The incidence of Hepatitis B Antigen (HBA) was 8.1% in Taipei district; 2.2% among the aged with good economy; 16.5% among the youth living in the area of bad sanitation. HBA was positive in 26.4% of infectious hepatitis and in 41.6% of post-transfusion hepatitis. Chronic hepatitis developed mostly in male (87.5%) and was associated with HBA in 53.4%, but with anti-nuclear factor only in 23.6%. The incidence of primary hepatocellular carcinoma of the liver was high in Taiwan, its male to female ratio was 8 to 1. In 73.7%, it was associated with postnecrotic cirrhosis. The age incidence had a peak between 41 to 50. It was associated with HBA in 11.5% by Quchterlony's double diffusion test, but was 75.7% by immune adherence hemagglutination test. Anti-HBA appeared in 8.7% by passive hemagglutination test which was much lower than that of other malignancies (33.3%). Aflatoxin was detected in many dried foods in Taiwan, but the content was low. (Author Abstract)

13. AFLATOXIN INHALATION AND ALVEOLAR CELL CARCINOMA.

Dvorackova I
Dept. Pathology, Charles Univ., Hradec Kralove, Czechoslovakia
Br Med J; 1(6011):691 1976

The case history of a 68-yr-old man who possibly inhaled aflatoxin and who died of pulmonary adenomatosis 14 mo later is presented. He had worked for 3 mo with peanut meal contaminated by the mold Aspergillus flavus. A colleague who had been doing the same work died of the same disease. It is hypothesized that the pathogenesis and etiology of primary pulmonary adenomatosis are due to congenital malformations and that the inhalation of irritant substances, along with other factors that lead to chronic lung damage, causes the disease. Apparently, inhalation of aflatoxin is dangerous.

14. MYCOTOXIN-PRODUCING FUNGI FROM HOUSE ASSOCIATED WITH LEUKEMIA.

Wray BB, O'Steen KG
Dept. Pediatrics, Medical Coll. Georgia, 1459 Gwinnett St., Augusta, GA 30902
Arch Environ Health; 30(12):571-573 1975

To determine if mycotoxin-producing fungi were present in the air, wood, or surroundings of a Southern Georgia house associated with four leukemic patients (3 occupants and 1 regular visitor), fungi sampling was performed. One hundred and ten strains of 21 common genera were identified. Of the 11 isolates tested in Peking ducklings, young male hamsters and BALB/c low-leukemia mice, only Aspergillus fusarium produced effects in all three. A parasiticus produced aflatoxin. Similar genera were isolated from non-leukemia houses with the exception of Trichoderma, Verticillium and Monotospora. It is suggested that certain mycotoxins may be related to the pathogenesis of leukemia, possibly as an expression of their immunosuppressive effects. (21 refs)

15. RELATION BETWEEN DIFFUSE ASPERGILLOSIS AND PULMONARY CARCINOMA IN A PATIENT WITH CHRONIC LYMPHOID LEUKEMIA.

Gordon IB, Gorbatovskaya NS, Rykov VA
First Dept. Therapy, Novokuznetsk Inst. Postgraduate Studies for Physicians, Novokuznetsk, USSR
Klin Med (Mosk); 54(8):133-134 1976

A 54-yr-old man with chronic lymphoid leukemia developed diffuse aspergillosis of the lungs, which was diagnosed by lung biopsy and sputum analysis. After the aspergillosis was checked by antibiotics, lung cancer was diagnosed by x-ray examination a few months later. The patient died of profuse pulmonary hemorrhage. Keratinizing squamous cell carcinoma of the wall of a cavern was found in the second segment of the left lung on autopsy. The aspergillosis is considered to have been facilitated by the impaired immune status caused by chronic lymphatic leukemia, and the carcinoma was probably due to the carcinogenic effect of the aflatoxins produced by Aspergillus. (7 refs)

16. PANCREATIC CANCER IN FATHER AND SON (LETTER TO EDITOR).

Reimer RR, Fraumeni JF, Ozols RF, Bender R
Environmental Epidemiology and Medicine Branches, NCI, Bethesda, MD 20014
Lancet; 1(8017):911 1977

The occurrence of a pancreatic tumor in successive generations is reported. A 63-yr-old chemical worker and his 36-yr-old son both developed pancreatic cancer. Several years before he died, the son worked briefly with his father, and both were exposed to vinyl chloride and other chemicals. Neither patient smoked or had any condition that predisposes to pancreatic cancer. The patients consumed large quantities of pistachio nuts, and they may have ingested some that were contaminated with mycotoxins. The occurrence of this tumor in successive generations may be due to chance or genetic transmission. Some characteristics of the case suggest an environmental influence. (8 Refs)

B. Other Human Diseases Related to Mycotoxin Exposure

17. EPIDEMIC OF HEPATITIS IN MAN DUE TO AFLATOXICOSIS.

Anonymous
No Affiliation Given
Nutr Rev; 34(2):45-46 1976

A summation of epidemiological studies suggests that the liver of humans is affected by aflatoxin. There is a correlation between primary liver cancer and aflatoxin exposure in Africa and Thailand. The symptoms of an epidemic in India were jaundice, rapidly developing ascites, portal hypertension, and a high mortality rate (many of which were due to massive gastrointestinal bleeding). Examination of the food showed that the maize was contaminated with Aspergillus flavus at 6.25 to 15.6 ppm. The epidemic was thus attributed to aflatoxin consumption despite the fact that neither aflatoxin B1 nor its metabolites were found in either liver or urine samples; it was found in only two of seven serum samples. (These data may not be valid, because the time lag between maize consumption and sample collection is unknown.) Histopathological examination showed extensive bile duct proliferation with periductal fibrosis and multinucleated giant cells. Men (sex ratio 2:1) and persons in age groups between 5 and 13 yr and over 30 yr were most afflicted. It would be pertinent to see if this community suffers from a high incidence of hepatoma in the future.

18. IMPLICATION OF MYCOTOXINS FOR HUMAN HEALTH.

Campbell TC, Stoloff L
Dept. Biochem. Nutr., Virginia Polytech. Inst. State Univ., Blacksburg
J Agric Food Chem; 22(6):1006-1015 1974

The implication of mycotoxins, especially aflatoxins, for human health is reviewed. It has been demonstrated that toxins in the absence of visible molds could produce effects (carcinogenesis and organ damage) after chronic exposure and at future times. Only a few cases of acute mycotoxicoses in humans have been recorded. The estimated risk to humans has been based on studies of various species of animals, especially primates, and on human incidents. In determining the risk to humans the authors consider comparative metabolic studies, primate studies, human incidents, and epidemiological studies. No conclusive statements can be made because of the many questions raised, i.e, genetic differences in susceptibility, natural selection, age and sex susceptibility, and metabolic differences. Other mycotoxins that have been implicated as harmful to human health are sterigmatocystin, patulin, penicillic acid, the trichothecene group, luteoskyrin, and citrinin.

19. AN AFLATOXIN IN THE LIVER OF A PATIENT WITH REYE-JOHNSON SYNDROME.

Chaves-Carballo E, Ellefson RD, Gomez MR
Univ. Iowa Coll. Medicine, Iowa City, Iowa
Mayo Clin Proc; 51(1):48-50 1976

The aflatoxin contents of liver specimens from 8 patients with Reye-Johnson syndrome, 2 patients with acute encephalopathy, and 10 patients without liver disease were analyzed. Aflatoxin was isolated from the liver of 1 patient, a 15-yr-old girl who had clinical, laboratory, and pathologic features of Reye-Johnson syndrome. Thin-layer chromatography, fluorescence emission, infrared spectroscopy, and derivatization studies characterized the aflatoxin as related structurally to, but not identical with, aflatoxin B1 . This report is added to previous ones from Thailand, New Zealand, and Czechoslovakia in which the association of aflatoxins and fatty liver has been found in Reye-Johnson syndrome.

20. URINARY EXCRETION OF AFLATOXIN BY NIGERIANS WITH ACUTE LIVER DISEASES (MEETING ABSTRACT).

Bababunmi EA, Francis TI, Bassir O, Rukari A
Dept Biochem, Univ Ibadan, Ibadan, Nigeria
Third International Symposium On Detection And Prevention Of Cancer; 480 1976

Because many foodstuffs which are sold in Ibadan markets are contaminated with measurable amounts of aflatoxin B1 (AFB) it appeared to us desirable to obtain information on the levels of AFB (or its metabolites) in the urine of three categories of the local adult male population: (a) normal healthy individuals, (b) hospitalized patients with acute liver diseases and (c) hospitalized patients who are not sufferers of any liver disease. Urinary excretion of a fluorescent compound has been detected in at least 90% of the persons in each category of the population. The fluorescence characteristics of this compound in CHC13-extract were similar to those of AFB. The relative fluorescence of AFB is quite high in 5mM-H2SO4 (pH 2). Fluorescence measurements of solvent extracts and beta-glucuronidase-hydrolyzed aqueous phases, in a medium of pH 2, indicated that normal individuals excrete quantities of AFB of the order of 0.45 mg per 24 hr. The relative excretion rates in the normal, no liver disease and liver disease sufferers were estimated to be in proportions 1:1:5.6 respectively. Cirrhosis and cancer profiles were similar. The possible role of aflatoxin poisoning in the aetiology of primary liver cancer is thus further demonstrated. (Author Abstract)

21. HEPATOCARCINOGENIC MATERIAL IN URINE SPECIMENS FROM HUMANS CONSUMING AFLATOXIN.

Campbell TC, Sinnhuber RO, Lee DJ, Wales JH, Salamat L
Dept. Biochem. Nutr., Virginia Polytech. Inst. State U., Blacksburg
J Natl Cancer Inst; 52(5):1647-1649 1974

Urine extracts from children who had consumed peanut butter contaminated with aflatoxin B1 (AFB1, 21 microg/child/2 days) induced hepatomas in 22 of 78 rainbow trout when fed for a period of only 30 days. The amount of AFB1 in the extracts was estimated to be equivalent to that contributed by the small quantity of AFM1 excreted. The data suggest that the major fraction of aflatoxin ingested by humans is not excreted as aflatoxins B1, M1 or P1 but as their relatively noncarcinogenic metabolites and conjugates.

II. EXPERIMENTAL CARCINOGENESIS AND AFLATOXICOSIS IN ANIMALS

A. Toxicity and Carcinogenesis by Mycotoxins in Rodents

22. CYCLOCHLOROTINE. (PP. 139-144)

Anonymous
IARC Monographs on the Evaluation of the Carcinogenic
Risk of Chemicals to Man. Some Naturally Occurring
Substances; International Agency for Res. on Cancer.
(Lyon, France, 7-13 October 1975): vol. 10, 1976

The chemical, physical, and carcinogenic properties of the mycotoxin, cyclochlorotine (islanditoxin) were reviewed. Of groups of 20 male ddNi mice fed 40 or 60 ug cyclochlorotine/animal/day for life, none developed liver cell tumors in the 40-ug groups, but those surviving the 60-ug dose for 200-700 days developed one differentiated liver carcinoma and two liver cell adenomas. Reticuloendotheliomas were found in one mouse given 40 ug and in two mice given 60 ug. No liver cell tumors or reticuloendotheliomas were observed in 19 male controls, 10 surviving 400 days and 2 surviving 700 days. The mouse LD50 's of cyclochlorotine were 0.33 mg/kg body weight iv, 0.47 mg/kg sc, and 6.55 mg/kg po. Cyclochlorotine toxicity in mice and rats is fatal in less than 24 hr in most cases. Toxicity induces respiratory and circulatory disturbances, convulsions, initial hyperglycemia, rapid fall in liver glycogen, decreased levels of succinic dehydrogenase, and inhibition of oxidative phosphorylation. Cyclochlorotine is a cirrhogenic agent causing fibrosis or cirrhosis of the periportal type.

23. GRISEOFULVIN. (PP. 153-161)

Anonymous
IARC Monographs on the Evaluation of the Carcinogenic
Risk of Chemicals to Man: Some Naturally Occurring
Substances; International Agency for Res. on Cancer.
(Lyon, France, 7-13 October 1975): vol. 10, 1976

The chemical, physical, and carcinogenic properties of the antifungal antibiotic griseofulvin were reviewed. Charles River mice were fed griseofulvin of particle size 0.41, 1.3, or 1.52 m^2 /g for 12-16 mo. Among mice fed 0.41 m^2 /g griseofulvin, 8/12 males and 0/13 females developed hepatomas; among those fed the other sizes, 11/11 males and 7/14 females developed hepatomas. Controls did not develop tumors. In another study with rats, of 11/20 survivors receiving 200 mg/kg body weight ip of a finely milled suspension of griseofulvin for 93 wk, 1 had an adenocarcinoma of the cervix. Male mice given sc injections of 0.5, 0.5, 1, or 1 mg griseofulvin (total dose 3 mg per animal) on days 1, 7, 14, and 21 of age developed hepatomas in 7/16 survivors by 49 wk compared with 4/48 controls. In a similar study with mice using the same dose and dosage schedule, only 24% developed hepatomas. Toxic liver necrosis was observed in mice fed 1-1.5 g/kg body weight/day griseofulvin for 82 days; rats and dogs exhibited no toxicity when fed 1-2 g/kg/day for 8 wk. Porphyria is induced in mice and rats. Griseofulvin acts as a cocarcinogen with skin applications of 3-methylcholanthrene in mice but not with benzo(a) pyrene. Malformed pups were produced by female rats receiving oral doses of 1,250 and 1,500 mg/kg/day from days 6 to 15 of pregnancy. Human tissue culture cells (EUE heteroploid line and a hybrid line derived from it) and phytohemagglutinin-stimulated human lymphocytes showed increased chromosome numbers distributed about tetraploidy in the presence of 20 and 60 ug/ml griseofulvin.

24. LUTEOSKYRIN. (PP. 163-169)

Anonymous
IARC Monographs on the Evaluation of the Carcinogenic
Risk of Chemicals to Man: Some Naturally Occurring
Substances; International Agency for Res. on Cancer.
(Lyon, France, 7-13 October 1975): vol. 10, 1976

The chemical, physical, and carcinogenic properties of the mycotoxin luteoskyrin (flavomycelin) were reviewed. Over a 328-day period groups of 10-30 male or female mice (ddNi or DDD) were fed 0, 50, 150, or 500 ug/animal/day purified luteoskyrin in a basal diet containing rice and/or barley. No tumors developed in controls or animals fed 50 ug luteoskyrin/mouse/day but five to eight liver cell adenomas and one or two liver cell carcinomas developed in groups of 29 or 30 animals fed 150 or 500 ug luteoskyrin/animal/day. Two liver cell adenomas and one liver cell carcinoma were found in mice fed the 500-ug dose intermittently every other 4-5 wk. Female mice were less sensitive. The minimum period for tumor development was 216 days. Luteoskyrin is also hepatoxic to rats, rabbits, and mice, although rabbits and mice are the most susceptible. The LD50 for male mice is 145 mg/kg body weight by sc injection, 221 mg/kg po, and 6.65 mg/kg by iv injection. The sc LD50 values in newborn mice were 7.2 mg/kg in males and 6.3 mg/kg in females. Luteoskyrin in the presence or absence of rat microsomes did not induce "unscheduled DNA repair synthesis" in cultured human fibroblasts.

25. OCHRATOXIN A. (PP. 191-197)

Anonymous
IARC Monographs on the Evaluation of the Carcinogenic
Risk of Chemicals to Man: Some Naturally Occurring
Substances; International Agency for Res. on Cancer.
(Lyon, France, 7-13 October 1975): vol. 10, 1976

The chemical, physical, and carcinogenic properties of ochratoxin A were reviewed. Oral administration of 100 or 300 ug/rat (males and females) for 5 days/wk for 50 wk resulted in one hamartoma of the kidney in the animal receiving the highest dose. Rainbow trout did not develop tumors when fed ochratoxin A for 8 mo at 16, 32, or 64 ug/kg of diet, but hepatomas were produced when sterculic acid was included in the diet. No tumors were observed in 7/20 survivors 81 wk after sc injection of 10 male and 10 female mice with 10 ug ochratoxin A in 0.1 ml arachis oil twice weekly for 36 wk. Local fibrosarcomas developed in 2/10 female Wistar rats given sc injections of 2.5 mg/kg body weight ochratoxin A in sunflower seed oil weekly for 18 wk; 2/10 controls (sunflower oil alone) also developed local fibrosarcomas. Acute oral LD50 's of ochratoxin A for rats were 22 mg/kg for males and 20 mg/kg for females. Nephropathy has been observed experimentally in pigs, dogs, rats, chickens, and trout. Increased prenatal mortality, decreased fetal weight, and fetal malformations were manifested by pregnant mice given one dose ip on days 7-12 of gestation.

26. PENICILLIC ACID. (PP. 211-216)

Anonymous
IARC Monographs on the Evaluation of the Carcinogenic
Risk of Chemicals to Man: Some Naturally Occurring
Substances; International Agency for Research on
Cancer. (Lyon, France, 7-13 October 1975); vol. 10, 1976

Some chemical and physical data and biological data on its carcinogenicity to man have been listed for the mycotoxin penicillic acid, which is produced by certain species of Penicillium and Aspergillus and is sometimes found as a contaminant in moldy corn and poultry feed. No data from human case reports or epidemiological studies are available regarding carcinogenicity, but sc inoculation of mice and rats resulted in the production of local sarcomas. Local tumors developed in 6/19 Tuck No. 1 strain mice treated twice weekly with 0.2 mg in arachis oil for up to 65 wk. Local sarcomas and fibrosarcomas developed in between 48-67 wk in 4/4 rats given 1 mg of the substance in 0.5 ml of arachis oil. The LD50's of penicillic acid in mice were 250 mg/kg iv and 600 mg/kg po. The compound is known to induce single- and double-strand breaks in the DNA of HeLa cells.

27. STERIGMATOCYSTIN. (PP. 245-251)

Anonymous
IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man: Some Naturally Occurring Substances; International Agency for Research on Cancer. (Lyon, France, 7-13 October 1975): vol. 10, 1976

Some chemical and physical data and biological data concerning its carcinogenicity to man have been listed for the mycotoxin sterigmatocystin, which has been found as a contaminant of country-cured hams, wheat, and green coffee beans. No data concerning carcinogenicity in man are available, but it was shown to be carcinogenic in rats following oral, topical, or sc administration. When Wistar rats were fed 0.15-2.25 mg/day for 52 wk, 39/50 developed hepatocellular carcinomas within 123 wk. Skin papillomas and carcinomas occurred in 10/20 rats given 1 mg of sterigmatocystin twice weekly for 70 wk by application to the shaved dorsal skin in dimethylsulfoxide or acetone; hepatocellular carcinomas also occurred in 12 rats. Local sarcomas occurred in 3/6 rats treated twice weekly with 0.5 mg of the substance (sc, in arachis oil) for 24 wk, one rat also developed a hepatoma and one rat a cholangioma. Sterigmatocystin has also been shown to be carcinogenic in mice following oral administration: 21/25 ICR mice of both sexes fed a diet containing 5 ppm sterigmatocystin for 58 wk developed pulmonary adenomas (compared to 4/37 in control mice); adenocarcinomas of the lung occurred in 9/25 treated mice, but in none of controls.

28. PATULIN: A MYCOTOXIN OF POTENTIAL CONCERN IN FOODS.

Stott WT, Bullerman LB
Dept. Food Science and Technology, Univ. Nebraska, Lincoln, Nebr. 68503
J Milk Food Technol; 38(11):695-705 1975

Patulin(4-hydroxy-4H-furo3,2 cpyran-2(6H)-one) is a metabolite of numerous Aspergillus and Penicillium species. It is toxic to some microorganisms, plants and animals, and carcinogenic to mice. It appears that man would be susceptible to the toxic effects of this compound. The possibility that patulin production could occur during refrigerated storage is real. However, the reactivity of patulin with sulfhydryl groups and other food components may cause its apparent disappearance from a food product. Apparently toxicity is lessened. Low pH or reactions with digestive enzymes might reverse these reactions or liberate free patulin in the gut. Certain foods such as cheeses and cured meats do not appear to support patulin production to any great extent. This, combined with the reactivity of patulin with sulfhydryl groups, seems to lessen the danger of patulin occurrence in these foods. However, apple products and grain products may pose more of a potential problem. Apple products are of particular concern since common apple rotting molds are frequent producers of patulin. Natural contamination with patulin has been found only in apple sap, juice, and cider (49-309 ppb). (143 refs)

29. TOXICITY TO WHITE MICE OF CORN NATURALLY CONTAMINATED WITH AFLATOXIN.

Lindenfelser LA, Lillehoj EB, Sansing GA
Res. and Development Div., Commercial Solvents Corporation, Terre Haute, Indiana 47808
J Agric Food Chem; 24(1):198-200 1976

The toxicity of corn naturally contaminated with aflatoxin was tested in CD-1 outbred albino mice. Male and female mice of 3 different ages (3, 5, and 8 wk old) were fed continuously on balanced corn diets containing aflatoxin. The corn contained 286 ppb aflatoxin and was fed in diets containing 5, 20, and 40% (14, 57, and 114 ppb) contaminated grain for 6 mo. Animals were observed daily for weight changes, general health, or toxicity symptoms, and at the end of the experiment their livers were examined for total lipid content. Weight gains of mice fed the highest aflatoxin levels were approximately the same as those on rations containing lower levels. No tumors were observed, but lipid accumulation in the liver was significant in animals ingesting the highest level of toxin-contaminated feed.

30. ROLE OF PODOPHYLLOTOXIN IN THE BEDDING AND DIETARY ZEARALENONE ON INCIDENCE OF SPONTANEOUS TUMORS IN LABORATORY ANIMALS. (LETTER TO EDITOR)

Schoental R
R. Veterinary College, Dept. Pathol., London, England
Cancer Res; 34(9):2419-2420 1974

The observation that in C3HA(vy)fB mice the high incidence of spontaneous mammary and hepatic tumor is related to a particular diet and bedding material is discussed relative to potential causative agents present. The lignan, podophyllotoxin, which is present in the red cedar wood shavings associated with high spontaneous tumor occurrence rates, has anti-mitotic activity and can cause hyperplastic lesions as well as having carcinogenic potential when fed to BALB/c mice. Likewise the possible presence of zearalenone, a natural estrogen produced as a secondary fungal metabolite and appearing as a common grain contaminant, is suggested as the carcinogenic factor present in the diet. While data is not yet available, a diethylstilbestrol-like effect on target organs is suggested. (19 refs)

31. LONG-TERM ADMINISTRATION OF STERIGMATOCYSTIN AND PENICILLIUM VIRIDICATUM TO MICE.

Zwicker GM, Carlton WW, Tuite J
Sch. Vet. Sci., Med., Purdue U., West Lafayette, Ind.
Food Cosmet Toxicol; 14(4):491-497 1974

The effects of long-term feeding of pure sterigmatocystin (S-p), sterigmatocystin in a rice culture of Aspergillus versicolor (S-rc), and/or a rice culture of Penicillium viridicatum (P-rc), were studied in white Swiss mice. The mice were fed a diet containing 5 ppm sterigmatocystin (S-p or S-rc) and/or a 7.5% content of P-rc for periods of 2 wk alternating with 2 wk periods on a control diet, for a total of 54-58 wk. In mice fed S-p or S-re alone, the incidence of pulmonary tumors were, respectively, 73 and 49% higher than that in the control group. The incidence of pulmonary tumors in mice fed P-rc was about 16% higher than that in the controls. In the groups fed P-rc with S-p or S-rc, the incidence of pulmonary neoplasms exceeded that in the controls by 54 and 52% respectively. The tumors were classified as adenomas or adenocarcinomas arising from the bronchial and alveolar epithelium. Adenomatosis was more common in mice fed diets containing sterigmatocystin, the incidence being 27-47% greater than that in controls. The incidence of other neoplasms was not affected by the feeding of the experimental diets. The increased incidence of pulmonary neoplasms in the mice fed S-p, S-rc or P-rc appeared to be directly related to the feeding of these test materials. The possible presence of certain common mycotoxins in the P-rc was eliminated by chemical analysis. It is concluded that P-rc and sterigmatocystin (S-p or S-rc) each had a carcinogenic effect when fed in the diet to mice but there is no additive effect when sterigmatocystin is combined with the P-rc diet.

32. AFLATOXIN AND TRICHOTHECENE TOXINS: SKIN TUMOR INDUCTION AND SYNERGISTIC ACUTE TOXICITY IN WHITE MICE.

Lindenfelser LA, Lillehoj EB, Burmeister HR
North. Regional Res. Lab., Agr. Res. Service, Peoria, Ill.
J Natl Cancer Inst; 52(1):113-116 1974

Toxic interactions of three naturally occurring mycotoxins were examined by the mouse skin tumor test and acute toxicity trials. Aflatoxin B1 (B1) functioned as a tumor-initiating substance; a single 25 microg dose applied before croton oil as promoter caused papilloma formation on more than half of eight animals. Neither Fusarium toxin, trichothecene-2 toxin (T-2) or diacetoxyscirpenol, served as initiating agents. These two mycotoxins acted as weak promoter substances on 7,12-dimethylbenz(a)anthracene (DMBA)-initiated mouse skin cells but not on mice treated with B1 as initiator. Either DMBA or B1 initiation followed by T-2 promotion (25 microg/dose) caused extensive skin damage and subsequent tolerance of the treated skin to elevated doses of T-2. In acute toxicity trials, combinations of B1 and T-2 produced a synergistic lethal response.

33. AFLATOXIN B1 -2,3-DICHLORIDE: A TOXIC AND REACTIVE DERIVATIVE OF AFLATOXIN B1 (MEETING ABSTRACT).

Swenson DH, Miller JA, Miller EC
U Wisconsin Med Cent Madison WI
Proc Am Assoc Cancer Res; 15:43 1974

Rodent liver microsomes convert aflatoxin B1 (AFB1) to its reactive 2,3-oxide which can be trapped as an RNA-AFB1 adduct (Swenson et al, BBRC 53: 1260; 1973). Isolation of the epoxide from enzymatic or chemical syntheses has not been possible. To obtain a reactive derivative of AFB1 with an electrophilic carbon-2 as a model for the epoxide, AFB1 was reacted with Cl2 to yield aflatoxin B1 -2,3-dichloride (AFB1 -Cl2), which was characterized by its uv and mass spectra and its solvolysis properties. The half-life of AFB1 -Cl2 in 5% aqueous acetone, pH 7.4, was 1.2 min. In 50% aqueous acetone at neutrality AFB1 -Cl2 bound covalently to a greater extent with cytidine than with guanosine or adenosine. No reaction was detected with uridine. Covalently bound adducts were also obtained on reaction with RNA, DNA, or protein. The skin of Charles River CD-1 mice was treated 2 times (1-wk interval) with AFB1 -Cl2 . At the 5th wk 2 times/wk applications of 0.5% croton oil were started. With total doses of 148 microg (0.4 micromoles) or 74 microg of AFB1 -Cl2 severe skin damage and a mortality of 14/25 or 4/25, respectively were seen by 6 wk. With 37 microg skin damage was less severe and the mortality was 1/25. At 8 wk skin papillomas were found as follows: 148 microg, 4 of 11 survivors with 10 papillomas; 74 microg, 6 of 21 survivors with 9 papillomas; 37 microg, 1 of 24 survivors with 2 papillomas AFB1 and AFB2 had no effect.

34. CARCINOGENIC EFFECTS OF LOW DIETARY LEVELS OF AFLATOXIN B-1 IN RATS.

Wogan GN, Paglialunga S, Newberne PM
Dept. Nutr., Food Sci., Massachusetts Inst. Technol., Cambridge
Food Cosmet Toxicol; 12(5/6):681-685 1974

The effects of low dietary levels of aflatoxin B1 were investigated in male Fischer rats. Aflatoxin B1 was added to a semi-synthetic diet at levels of 1, 5, 15, 50 and 100 microg/kg (ppb) and fed to the rats beginning when their body wt were 80 g and continuing for up to 105 wk. Hepatocellular carcinomas were induced in a high incidence by levels of 50 and 100 ppb (in 20 of 25 and 28 of 28 rats, respectively), and in lower incidence by the other dietary levels. Tumors were induced in 2 of 22 animals fed 1 ppb, and pathological lesions were present in the livers of 7 of the remaining survivors. It is concluded that aflatoxin B2 has demonstrable carcinogenic activity for rat liver at dietary levels as low as 1 ppb.

35. CEREBRAL GRANULAR CELL TUMOR IN THE RAT AFTER INTRAPERITONEAL APPLICATION OF AFLATOXIN B1 DURING PREGNANCY.

Tschahargane C, Georttler K, Ule G, Volk B
D-6900 Heidelberg, Postfach 104340, West Germany
Acta Neuropathol (Berl); 32(4):281-285 1975

A granular cell tumor measuring 5 x 4 x 4 mm found in the right frontal pole of the cerebrum in a rat that had been administered aflatoxin B1 (0.5 mg/kg/day, ip) between the 18th and 21st days of pregnancy 12 mo before spontaneous death due to the tumor was described. The tumor originated from the leptomeninx. The light and electron-microscopic findings and the nucleic acid concentrations justify a comparison with the Abrikossoff tumor. The protein content of the granules of the tumor cells was higher than that known for granular cell myoblastoma of man. No tumor was found in other organs. The offspring of the aflatoxin B1-treated female was normal. The granulocytic tumor, possibly induced by aflatoxin B1 in the rat, may serve as an experimental model for the study of this rare neoplasm.

36. INDUCTION OF LIVER TUMORS BY LOCAL IMPLANTATION OF CARCINOGENS (MEETING ABSTRACT).

Atermann K, Yuce G
Pathologisches Institut, Dalhousie Univ., Dalhousie, Nova Scotia, Canada
Verh Dtsch Ges Pathol; (59):482 1975

Liver tumor induction was studied in young rats and mice following the implantation in the liver of cylinders of pure carcinogens cholesetrol, anthracene, a-naphthylisothiocyanate (ANIT), butter yellow, 3-methylcholanthrene (3-MC), and aflatoxin. The experiment was conducted until spontaneous death of the animals. Cholesterol and anthracene induced no tumors. ANIT led to the formation of a very thick, nearly armorlike capsule and, finally, to a sarcoma with local osteogenesis. Aflatoxin induced sarcomas most likely from the capsule after a rather short latency time, as well as intestinal carcinomas, which indicates the carcinogenic effect of its metabolites. 3-MC induced numerous malignant tumors with sarcomatous, hemangiosarcomatous, and cholangiocarcinomatous elements. The squamous epithelial aplasia of the biliary epithelium led to keratinizing carcinomas. This is the first case ever reported on carcinoma induction in the intrahepatic bile ducts by locally applied 3-MC. Butter yellow induced hepatocellular carcinomas in many animals. Lung metastases were found in some animals. The hepatoma induction rate was lower after butter yellow implantation following the application of 3-MC. (No refs)

37. AFLATOXICOSIS IN RATS FED LOW LEVELS OF AFLATOXIN EITHER CHEMICALLY PURE OR AS A NATURAL CONTAMINANT (MEETING ABSTRACT).

Tisdel MO, Nees PO, Harris DL, Mann GE
WARF Inst., Inc., Madison, WI
Toxicol Appl Pharmacol; 41(1):166 1977

The present study was initiated to clarify the conflicting reports that ground roasted peanuts in the diet provide some dietary protective action against aflatoxin (AF). AF at 0, 5, 15, 50, and 200 ppb in a semisynthetic basal diet, a diet containing peanut butter, or a diet containing cottonseed meal was fed to Fischer strain rats for up to 104 wk. The sources of AF were a chemically pure supply and naturally contaminated peanuts and cottonseed meal. The presence of chemically pure AF in the basal diet or diet containing AF-free peanut butter resulted in a dose-related aflatoxicosis including carcinomas and/or hepatomas in animals receiving as little as 5 ppb. AF from naturally contaminated peanuts or cottonseed meal resulted in dose-related effects comparable to those produced from chemical AF. Tumor incidence was equal or greater at all AF levels in groups receiving naturally contaminated peanuts. Peanuts in the diet did not significantly alter the dose related effect. The data from this study indicate that a dose-related aflatoxicosis was due to the presence of AF in the total diet. Aflatoxicosis was not related to the source of AF, and there was no protective action against aflatoxicosis from the presence of peanuts or cottonseed meal. (no Refs)

38. HEPATIC CELL LOSS AND PROLIFERATION INDUCED BY N-2-FLUORENYLACETAMIDE, DIETHYLNITROSAMINE, AND AFLATOXIN B1 IN RELATION TO HEPATOMA INDUCTION.

Nishizumi M, Albert RF, Burns FJ, Bilger L
Dept. Public Health, Faculty Medicine, Kyushu Univ., Fukuoka 812, Japan
Br J Cancer; 36(2):192-197 1977

Three hepatic carcinogens (aflatoxin B1, diethylnitrosamine (DEN) and N-2-fluorenylacetamide (FAA)) were compared for carcinogenicity, early cell toxicity and parenchymal cell proliferation. The carcinogens were administered to rats for 15 wk as follows: aflatoxin B1, 1 in 10^6 in pelleted food; DEN, 2 in 10^4 in drinking water; FAA, 3 in 10^4 in pelleted food. The loss of prelabeled DNA and the H3-thymidine pulse-labeling indices of parenchymal and nonparenchymal cells were determined at various times during the period of carcinogen availability. On a molar basis, aflatoxin B1 was 90 times as carcinogenic as FAA and 24 times as carcinogenic as DEN. However, for about equal magnitudes of hepatic cell proliferation and loss, aflatoxin B1 was the least potent carcinogen. For a given level of carcinogenicity, FAA was more potent than DEN in causing loss of hepatic DNA and in increasing the parenchymal cell labeling index. DEN and aflatoxin B1 produced about the same degree of DNA loss and parenchymal cell labeling, but the former was a more potent carcinogen. When carcinogenicity was compared for approx equal levels of early hepatic cell destruction and proliferation, the three chemicals in the present study could be ranked in descending order of potency as DEN, FAA and aflatoxin B1. (Author abstract) (11 Refs)

39. A NEW METASTASIZING HAMSTER TUMOR INDUCED BY SHORT-TERM EXPOSURE TO AFLATOXIN IN VITRO (MEETING ABSTRACT).

Jones RF, Thornton J, Fagan V, Vellios F
U. Texas Southwestern Med. Sch., Dallas
Proc Am Assoc Cancer Res; 15:106 1974

This study was undertaken to test the ability of aflatoxin to produce rapid transformation of hamster embryo cells in vitro. Primary hamster embryo cultures were exposed for 48 hr to aflatoxin B1 at concentrations of either 0.1 or 0.05 microg/ml. The cultures were carried in tissue culture by weekly transfers for 12 to 16 wk, at which times morphologic changes, rapid growth rates, and high cell densities indicated cell transformation. The cell lines were then implanted into newborn hamsters and subcutaneous tumors appeared within 4 wk. Ninety-six percent of animals developed fibrosarcomas at the site of inoculation and 2 litters developed lymphatic and pulmonary metastases. Three separate transformed cell lines occurred and each transformation event was proved by the appearance of tumors in vivo. Examination of the cell lines by immune fluorescence, reverse transcriptase test, and the tritiated uridine test for type C particles yielded no evidence of contaminating oncogenic viruses.

B. Toxicity and Carcinogenesis by Mycotoxins in Other Species

40. TOXICOLOGICAL STUDY OF PATULIN IN MONKEYS.

Garza HC, Swanson BG, Branen AL
Dept. Food Science and Technology, Washington State Univ., Pullman, WA 99164
J Food Sci; 42(5):1229-1231,1235 1977

Patulin, 4-hydroxy-4H-furo 3,2-c- pyran-2(6H)-one, a mycotoxin produced by certain Aspergillus and Penicillium species, has been found as a contaminant of apple products. Patulin has resulted in toxicological effects in mice and rats; however, little information is available on the po toxicity of patulin to primates. In this study, pig-tail monkeys (Macaca nemestrina) were given daily po doses of patulin for a period of 4 wk. Four groups of two monkeys each were used. One group served as a control and the other groups were given daily dosages of either 5, 50 or 500 microg of patulin per kg of body wt. After 4 wk, the monkeys on 500 microg/kg were given daily dosages of 5 mg/kg for an additional 2 wk. To administer appropriate dosages, patulin was dissolved in citric acid buffer and applied to sliced freeze-dried bananas. Blood samples were taken weekly and hematology, serum protein electrophoresis and levels of serum glutamate-oxalacetate transaminase, alkaline phosphatase, blood urea nitrogen, cholesterol, protein, glucose, sodium and potassium were determined. No statistically significant changes were observed in these physiological parameters, with the exception of alkaline phosphatase, which decreased as patulin dosages increased. (Author abstract) (29 Refs)

41. INDUCTION OF CHOLANGIOCARCINOMA FOLLOWING TREATMENT OF A RHESUS MONKEY WITH AFLATOXIN.

Tilak TB
Natl. Inst. Nutr., Hyderabad, India
Food Cosmet Toxicol; 13(2):247-249 1975

Carcinogenic activity of aflatoxin was observed in a monkey treated with the substance over a long period. A female rhesus monkey weighing 2.0 kg was treated with a crystalline preparation of mixed aflatoxins (B1 44%, G1 44%, and B2 and G2 2%) for 5.5 yr. The preparation was administered im five days/week for the first yr in a dose of 50 microg/day for one month and 100 microg/day thereafter. A dose of 100 microg/day was given po on five days/week for the remaining 54 months. A massive globular greyish white hard mass occupying the lateral portion of the right lobe of the liver was observed at autopsy and was attached to the diaphragm. The weight of the liver with the tumor was 465 g. There were satellite nodules about 1 cm in diameter. Enlargement of lymph nodes in the porta hepatis was observed. On the basis of the morphology, the tumor was designated an adenoacanthoma of the intrahepatic bile ducts, or possibly an adenosquamous cholangiocarcinoma. The tumor was presumably induced by aflatoxin for the following reasons: a) aflatoxin is one of the most potent hepatocarcinogens known, in lower animals, cholangiocarcinomas constitute one of the known sequelae of chronic aflatoxicosis, and b) this type of tumor does not ordinarily arise spontaneously in monkeys. The author suggests that aflatoxin consumption may be related to human primary liver cancer in some areas,(Swaziland and Thailand), and recommends the review of safe amounts of these toxins in human food.

42. HORMONE EFFECTS OF ZEARALENONE IN NONHUMAN PRIMATES.

Hobson W, Bailey J, Fuller GB
International Center Environmental Safety, Albany
Medical Coll., Holloman Air Force Base, NM 88330
J Toxicol Environ Health; 3(1/2):43-57 1977

A rhesus-monkey model based on gonadotropin changes in castrated animals exposed to estrogenic compounds is described and is used to compare the oral and parenteral potency of zearalenone (Z). The ability of Z to alter the levels of luteinizing hormone or follicle-stimulating hormone was compared with that of estradiol-17-beta (E) and diethylstilbestrol (DES). The level at which the experimental model can be used to detect injected E or DES is similar to that of rodent models using mice and rats. When given in a sc injection, Z is only slightly less potent than E or DES, but when taken orally, the potency of Z is significantly reduced. The action of Z on the hypothalamus or pituitary does not appear to be different than that of other estrogens. Z had no detectable antiestrogenic activity. The rhesus monkey can be used as a model for predicting the consequences of low-level exposure to Z for humans. (23 Refs)

43. CARCINOGENIC RESPONSE OF BROOD SOWS FED AFLATOXIN FOR 28 TO 30 MONTHS.

Shalkop WT, Armbrecht BH
Div. Vet. Res., FDA, Beltsville, Md.
Am J Vet Res; 35(5):623-627 1974

Four brood sows were fed aflatoxicogenic cultures and an aflatoxin concentrate in the basal ration for 28 to 30 months. The dose rates of aflatoxins B1 and G1 were 35 microg/kg body weight/day during growth period (8 months), 10 microg/kg/day during gestation, and 40 microg/kg/day during lactation (6 weeks). One sow, used as a control, received basal ration only. The only clinical sign in the 4 treated sows was a reduction in weight gain. Macroscopic changes involved only the liver in 3 of the 4 sows. Microscopic changes included hyperplastic nodules, as large as 6mm diameter, in the livers of all treated sows. These nodules were composed of relatively normal hepatic cells, but without central veins or bile ducts. Nodular hepatocellular adenomas were commonly seen. In 1 sow there were metastases to the omentum by seeding of hepato-cellular nodules and also intravascular metastases of undifferentiated neoplastic cells to the hepatic lymph nodes. Varying degrees of cirrhosis were noted in 3 of the sows, and was characterized in one by bile duct proliferation, fibrosis, and nodular hyperplasia, with many of the nodules undergoing central anoxic necrosis. Intravascular hepatic cell emboli were noted in livers of 3 sows. The emboli were degenerating in most instances. It is concluded that the hog requires a longer period of exposure to the carcinogenic effect of aflatoxin to produce hepatic cell carcinomas with systemic involvement by metastasis.

44. LONG TERM EFFECTS OF AFLATOXIN B1 AND VIRAL HEPATITIS ON MARMOSET LIVER. A PRELIMINARY REPORT.

Lin JJ, Liu C, Svoboda DJ
U. Kansas Sch. Med., Kansas City, KA
Lab Invest; 30(3):267-278 1974

Marmosets (36) were divided into 4 groups with group 1 receiving a diet containing 200 microg/kg aflatoxin B1, group 2 receiving the aflatoxin B1 diet plus virus injections containing hepatitis agent 6 weeks after the start of the diet, group 3 receiving only the hepatitis agent, and group 4 serving as controls. Long term observation of the effects of aflatoxin B1 and viral hepatitis on marmoset liver in terms of morphologic and chemical changes were noted. Viral hepatitis alone did not induce liver cirrhosis and/or hepatic tumors in nonhuman primates, but did result in occasional stellate portal fibrosis of the liver. In aflatoxin B1 injury, marmosets had cirrhosis in addition to hepatic tumors. The double injury of aflatoxin B1 and viral hepatitis produced more severe effects on marmoset liver than when the animals were injured with a single agent. Cirrhosis was much more severe, but transaminase elevation did not show an additive affect with double injury in the acute stage. Hepatic injury was parallel to the dose of aflatoxin B1. Males were more sensitive to aflatoxin injury. Ultrastructural changes of hepatocytes were reversible on withdrawal of aflatoxin. Hepatic tumors appeared late in these studies. Tumors were histologically indistinguishable from malignant liver tumors induced by aflatoxin in rats; therefore, it is believed that, if the marmoset had lived longer distant metastasis could have developed in some of these animals.

45. INDUCTION OF LIVER TUMORS BY AFLATOXIN B1 IN THE TREE SHREW (TUPAIA GLIS), A NONHUMAN PRIMATE.

Reddy JK, Svoboda DJ, Rao MS
Dept. Pathology, Univ. Kansas Medical Center, Kansas
City, KA 66103
Cancer Res; 36(1):151-160 1976

Aflatoxin B1 was administered to male and female tree shews (Tupaia glis) to ascertain its carcinogenicity in nonhuman primates. The carcinogen was administered in the diet at 2 ppm to 10 female and 8 male shrews. Of 12 animals that survived, 6/6 females and 3/6 males developed hepatocellular carcinoma between 74 and 172 wk after the beginning of the experiment. None of the eight control animals developed cancer of the liver. The estimated total aflatoxin B1 consumption ranged from 24 to 66 mg. Considerable variation in hepatocellular responses were noted: in two shrews, the tumors were associated with severe postnecrotic scarring while in the other seven, only mild to moderate portal fibrosis was encountered. These differences were attributed to inherent differences in the susceptibility within a given species of outbred animals. The results suggest extreme caution in proposing the permissible or safe levels of contamination of carcinogens in food. This study also emphasizes the usefulness of the tree shrew for long-term carcinogenic studies. (27 refs)

46. EXPERIMENTAL ACUTE AFLATOXICOSIS IN MINK (MUSTELA VISON).

Chou CC, Marth EH, Shackelford RM
Dept. Food Sciences, Food Res. Inst., Univ. Wisconsin-
Madison, Madison, WI 53706
Am J Vet Res; 37(10):1227-1231 1976

The suceptibility of mink (Mustela vison) to aflatoxin intoxication was studied. Mink were given single po doses (300, 500 or 900 microg aflatoxins B1 and G1/kg body wt). One, two and four minks in each group of five died within 4 days of ingesting the small, medium, and large doses, respectively. The most common lesion in the dead minks was an enlarged liver with pale yellow to yellowish-pink spots. Some lobules of the liver appeared hemorrhagic and fragile, while others had fatty metamorphosis. There were different degrees of fat infiltration, bile duct proliferation, and necrosis of liver cells. Minks that survived were not significantly different from control minks in terms of Hb content, packed cell vol, and WBC count. The liver specimens from the aflatoxin-treated minks contained more fat, were larger, and contained less protein, RNA, and DNA than did specimens from control minks. Aflatoxin residue (only B1) was recovered from liver of six minks and was between 0.1 and 7.6 microg of the original dose. (27 Refs)

47. IMMUNOSUPPRESSION IN CHICKENS BY AFLATOXIN.

Thaxton JP, Tung HT, Hamilton PB
Dept. Poultry Sci., North Carolina St. U., Raleigh
Poult Sci; 53(2):721-725 1974

Aflatoxicosis was induced in male broiler chicks by incorporating aflatoxin (0, 0.625, 1.25, 2.5, 5.0, and 10 microg/g) in the diet from hatching. At 24 days of age, the birds were immunized with sheep red blood cells (SRBC). All concentrations of aflatoxin significantly depressed the formation of hemagglutinin (HA) 3, 6, and 9 days after SRBC injection, the degree of immunosuppression being directly related to the dietary aflatoxin concentration on days 3 and 6. In the birds fed the lower aflatoxin concentrations, the antibody levels reached a maximum 6 days after SRBC injection, decreasing thereafter. In the birds fed the 5 and 10 microg levels, the antibody levels did not reach their maximum until 12 days after SRBC injection. Initiating the feeding of aflatoxin at the same time that the antigen was injected gave similar though more variable results. The relative sizes of the bursa of Fabricius and the thymus were reduced by dietary aflatoxin. The stage of immunosuppression can account for the carcinogenicity of aflatoxin as well as the enhanced susceptibility to some infectious agents found during aflatoxicosis.

48. THE CARCINOGENICITY OF AFLATOXIN M1 IN RAINBOW TROUT.

Canton JH, Kroes R, Van Logten MJ, Van Schothorst M, Stavenuiter JF, Verhulsdonk CA
Lab. Toxicology, Natl. Inst. Public Health, Bilthoven, The Netherlands
Food Cosmet Toxicol; 13(4):441-443 1975

Three groups, each initially of 250 rainbow trout, were fed a diet containing 5.8 ppb aflatoxin B1 (AFB1) or 5.9 or 27.3 ppb aflatoxin M1 (AFM1) for 16 mo. Toxin administration had no effect on the serum and liver enzymes studied and growth was unaffected up to mo 12, although at mo 15 there was a significant decrease in body wt in the two groups given AFM1 . Autopsy of fish killed after 5, 9 and 12 mo revealed ceroid degeneration of the liver in all 4 groups but no tumors or preneoplastic changes. Autopsy of survivors at mo 16, however, revealed 6 with hepatocellular carcinoma and 11 with hyperplastic nodules in the 48 trout fed 5.8 ppb AFB1 and 1 with hepatocellular carcinoma and 3 with hyperplastic nodules in the 48 fed 27.3 ppb AFM1 , but no evidence of such lesions was seen in the 49 fed 5.9 ppm AFM1 or in the 51 from the control group. The results indicate that AFM1 is a less potent carcinogen for trout than AFB1 .

49. NUTRITIONAL FACTORS IN TROUT HEPATOMA.

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Fish Disease Lab., Experimental Inst. Animal Prophylaxis in Piedmont and Liguria, Via Bologna 148, Torino, Italy
Prog Exp Tumor Res; 20:317-338 1976

Available knowledge on trout hepatoma is reviewed with particular emphasis on the most widely accepted etiological hypothesis; i.e the nutritional factor. The trout species most typically affected by liver tumors is the rainbow trout (Salmo gairdneri) under hatchery conditions. Signs and lesions characterizing trout hepatoma are obvious, and presumptive diagnosis seldom requires histological confirmation. The tumor apparently starts as a regenerative, hyperplastic nodule consisting of larger-than-normal cells with basophilic cytoplasm. A number of studies have proved that aflatoxin B1 is the causative agent of rainbow trout hepatoma. Some ingredients, such as peanut and cottonseed meals, used in the formulation of modern trout pellets are recognized as the foods most frequently contaminated by aflatoxins. A dry diet that contained no peanut or cottonseed meals and was aflatoxin-negative at manufacturing time has also induced hepatomas in trout. Such a diet became slightly aflatoxin-positive during long storage periods (7-12 mo), indicating that trout pellets, as well as ingredients for any trout feeding, must be freshly prepared and kept under proper storage conditions. Since the discovery of its nutritional etiology, trout hepatoma is gradually disappearing. The trout's high susceptibility to aflatoxin hepatomagenesis makes it extremely useful in identifying tiny amounts of aflatoxins in food products consumed by both animals and humans. (64 refs)

50. EXPERIMENTAL CARCINOGENESIS IN SMALL AQUARIUM FISHES.

Matsushima T, Sugimura T
Dept. Molecular Oncology, Inst. Medical Science, Univ. Tokyo, Minato-ku, Tokyo 108, Japan
Prog Exp Tumor Res; 20:367-379 1976

Chemical carcinogenesis studies in small aquarium fish, especially in zebra danio (Brachydanio rerio) and guppy (Lebistes reticulatus), are reviewed. Liver tumors have been induced in both species by N-nitrosodiethylamine and in guppies by N-nitrosodimethylamine, N-2-fluorenylacetamide, 4-dimethylaminoazobenzene, and o-aminoazotoluene. 3-Methylcholanthrene and benzo(a)pyrene induced epitheliomas in the stickle-back, Gasterosteus aculetus, and the bitterling, Rhodeus amarus, but not in the carp, Cyprinus carpio, after painting on the skin. Inoculation of G aculetus with benzo(a)pyrene did not induce tumors during a 4-mo observation period. Hepatomas were induced in guppies by feeding them a diet containing 6 ppm aflatoxin B1 for 9-11 mo. Guppies developed cholangiomas when given a diet containing 0.002% sterigmatocystin for 57 days, indicating that they are apparently more sensitive to the carcinogenic action of this compound than to aflatoxin B1. Rainbow trout also developed hepatomas when fed a diet of aflatoxin B1. After exposure of immature guppies to cycasin, methylazoxymethanol-b-glucoside, in the water, acute degenerative changes were seen in the liver and pancreas. When B rerio were given a diet supplemented with 50% of cycad nut meal from Cycas circinalis for 44 wk, many foci of malignant neoplasms developed in the liver. Orally administered bracken produced intestinal hyperplasia in guppies. A direct correlation has been observed between exposure to UV radiation and tumor incidence in fish. The carcinogens that produced hepatomas in fish are all known to be activated metabolically to proximate or ultimate carcinogens in the liver of mammals, indicating that fish liver has the same metabolic system as mammalian liver for activating carcinogens. The use of small aquarium fish has several advantages over the use of test rodents in studies of chemical carcinogenesis; i.e., fish are more sensitive to test carcinogens than rodents, they are more resistant to the toxic effects of test chemicals than mammals, no special facilities other than those in an ordinary laboratory are needed, large numbers of fish can be raised and maintained easily, and potential environmental carcinogens can be more quickly and easily detected in fish than in mammals. (27 refs)

51. ACUTE AFLATOXICOSIS IN FISH - INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON THE ULTRASTRUCTURE OF THE LIVER CELL (MEETING ABSTRACT). (P. 72)

Ishikawa T, Yamaguchi K, Yokoyama T, Takayama S
GANN Proceedings of the Japanese Cancer Association, 33rd Annual Meeting, October, 1974. Japanese Cancer Association Sendai Japan, 1974.

Since aflatoxin B1 administered in small doses over a long period of time causes hepatocarcinoma and since fish are cold-blooded animals, consideration was given to the effect of environmental temperature on the carcinogenesis. Wakin goldfish were studied at two environmental temperatures (15 and 25 C) for differences in LD50 value of aflatoxin B and in ultrastructure of the liver following ip injection with one 2.6, 8, or 25 mg/kg dose of the agent. The fish were dissected 1, 3, 7, or 21 days later, and histologic and electron microscopic examination of their livers was performed. Individual fish survived the poisoning for up to 5-8 mo. The LD50 value at 21 days was estimated to be 8 mg/kg at 25 C and approx 2x that at 15 C. Histologically, liver cell damage and regeneration required a longer time at the lower temperature than at the higher temperature. With the aid of the electron microscope, segregation of nucleoli and proliferation of endoplasmic reticulum were observed and were noted to be similar to those which occur in mammals. These reactions varied slightly with each temperature group. (no refs)

52. TOXICITY AND MORPHOPATHOLOGIC EFFECT OF AFLATOXIN IN FROG TADPOLES.

Puscariu F, Papillian VV, Gabor M, Deac C
Inst. Public Health Med. Res., Cluj, Roumania
Arch Roum Pathol Exp Microbiol; 32(2):255-267 1973

The LD50 of AT-B1 aflatoxins on Bombina sp and Rana temporaria tadpoles was determined by exposing the tadpoles to solutions of varying concentrations of aflatoxins and observing the mortality rate at different times. The differences in toxicity for the two species were not significant. The LD50 for two-week-old tadpoles after 36 and 48 hr resp were 1.9 and 1.6 mg/l, and for tadpoles aged 2-4 weeks the LD50 after 36 and 48 hr resp were 2.2 and 1.8 mg/l AT-B1. From these results, the MTD of aflatoxins in food is calculated as .015-.023 microg AT-B1/kg taking into account a safety coefficient of 100. Tadpoles exposed to sublethal doses of aflatoxina in concentrations of 2-6 microg/ml showed retarded development and metamorphosis and a swelling of the posterior part of the body proportional to the dose of aflatoxin. They also developed only rudimentary hind legs. After five weeks the surviving tadpoles began to develop a clear ascites containing a small number of cells which gradually increased with time. The cells were mostly nucleate RBC, but included also large cells similar to endothelial, lymphoid, and epithelial cells.

53. MYCOTOXICOSES.

Bojan F
Debreceni Orvostudomanyi Egyetem, Kozegeszsegtani es Jarvanytani Intezet, Debrecen, Hungary
Orv Hetil; 118(33):1961-1964,1967 1977

Hygienic and toxicological aspects of mycotoxins are reviewed. Aflatoxins were found to induce tumors of the liver, lungs, esophagus, stomach, duodenum, colon, and kidney in experimental animals. A relationship was found between the aflatoxin uptake and the incidence of liver cancer. (22 Refs)

C. Modification of Carcinogenic Action of Mycotoxins

54. EFFECT OF DIETARY SODIUM SELENITE UPON LESIONS INDUCED BY REPEATED SMALL DOSES OF AFLATOXIN B1 (MEETING ABSTRACT).

Grant KE, Conner MW, Newberne PM
Dept. Nutrition and Food Science, Massachusetts Inst. Technology, Cambridge, MA
Toxicol Appl Pharmacol; 41(1):166 1977

The protective effect of dietary sodium selenite against the effects of an acute dose of aflatoxin B1 was studied in 140 male Sprague-Dawley rats fed diets containing 0.03, 0.10, 0.50, 1.00, 2.50, or 5.00 ppm selenium as sodium selenite in a normal or marginally lipotrope-deficient semisynthetic basal diet. Each animal was given 25 microg of aflatoxin B1 po 5 days/wk for 4 wk. After 17 mo, histopathology revealed a very low incidence (20%) of hepatocarcinomas in animals receiving 1.0 ppm selenium in the normal basal diet and 0.1 and 0.5 ppm in the deficient basal diet. No other tumors were observed, and grading of the hepatic lesions indicated no significant difference among dietary selenium groups. Renal lesions were more striking. Large bizarre tubule cells were noted in relatively normal tubules surrounding areas of nephrosis. Nephrosis increased in severity with increasing dietary selenium concentration. Tissue selenium concentration and erythrocyte-glutathione peroxidase activities varied with dietary selenium but were lower in lipotrope-deficient animals than in normal basal diet animals. Dietary sodium selenite and repeated doses of aflatoxin B1 interact to produce large bizarre cells in the renal tubules. This lesion occurs in the region of the kidney where severe necrosis was noted previously in sodium selenite-fed animals given an acute dose of aflatoxin B1. There was no significant change in aflatoxin-induced hepatic lesions. (no Refs)

55. COMBINATION EFFECT OF CITRININ AND OTHER CHEMICALS ON RAT KIDNEY TUMORIGENESIS.

Shinohara Y, Arai M, Hirao K, Sugihara S, Nakanishi K, Tsunoda H, Ito N
First Department of Pathology, Nagoya City University Medical School, and Faculty of Education, Gunma University
Gann; 67(2):147-155 1976

Histological studies were made on the nephrotoxic effect of citrinin on the kidneys of rats, with or without previous treatment with the nephrotoxic chemicals, N-(3,5-dichlorophenyl) succinimide (NDPS) and N-nitrosodimethylamine (DMN). Oral administration of 0.02% or 0.05% citrinin alone caused signs of kidney injury but did not induce kidney tumors. On treatment with DMN alone, 8 of 14 rats (57.1%) developed kidney tumors; two (14.3%) were renal cell tumors, eight (57.1%) embryonal cell tumors, and one (7.1%) hemangioendothelioma. On the other hand, kidney tumors developed in 18 of 19 rats (94.7%) and 13 of 15 rats (86.7%) by the administration of 0.02% and 0.05% citrinin, respectively, after DMN. The tumors in these two groups were diagnosed histologically as renal cell tumors in 18 (94.7%) in group IV and 13 (86.7%) in group III, and as embryonal cell tumors in 14 (73.7%) in group IV and 9 (60.0%) in group III. Thus, in groups treated with citrinin after DMN the incidence of renal cell tumors was much greater and the incidence of embryonal cell tumors slightly greater than in the group treated with DMN alone. Kidney tumors developed in 4 of 18 rats (22.2%) treated with 0.02% citrinin after NDPS, but treatment with NDPS alone did not induce kidney tumors. Thus, treatment with citrinin changes the histological type and incidence of kidney tumors in rats induced by DMN. Moreover, this study confirms that citrinin in combination with NDPS can induce kidney tumor in rats, which was renal cell tumor (adenoma) histologically. (Author Abstract)

56. EFFECT OF VARYING LEVELS OF DIETARY PROTEIN ON TUMOR DEVELOPMENT AND LIPID METABOLISM IN RATS EXPOSED TO AFLATOXIN.

Wells P, Aftergood L, Alfin-Slater RB
Sch. Public Health, Univ. California, Los Angeles, Los Angeles, CA 90024
J Am Chem Soc; 53(9):559-562 1976

To determine the relationship of protein nutrition to aflatoxicosis, the effects of low (8%), normal (22%), and high (30%) protein-containing diets on tumor incidence and development and on several chemical indices were studied in USC weanling rats exposed to low levels of aflatoxin. Wt gain was markedly decreased and liver wt increased in all aflatoxin-fed rats but those on the low protein diets, who showed no changes. The low-protein, aflatoxin rats had no liver tumors. The severity of the liver involvement in the other rats increased with increased protein diet. When cystine was included in the diet, even the low-protein rats developed tumors. The livers of those animals on normal and high protein diets were much more severely involved than were the livers of animals on non-cystine supplemented diets. Plasma cholesterol levels were increased in response to aflatoxin when the diets containing 22% and 30% protein were used and when cystine was included in the 8% diet. Liver cholesterol levels were higher in all aflatoxin groups except the low protein one, which had no change. Aflatoxin induced changes in the fatty acid composition of sterol esters, triglycerides, and the phospholipids of liver and tumor tissue. (22 Refs)

57. VARIABLE EFFECTS OF A LIPOTROPE-DEFICIENT, HIGH-FAT DIET ON CHEMICAL CARCINOGENESIS IN RATS.

Rogers AE
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Cancer Res; 35(9):2469-2474 1975

Earlier studies demonstrated enhanced chemical carcinogenesis in the liver, colon, and probably esophagus of male rats that were fed a lipotrope-deficient, high-fat diet. In further experiments, designed to examine the range of the dietary effect on chemical carcinogenesis, rats were fed either the marginally lipotrope-deficient, high-fat diet or an adequate control diet, and treated with N-2-fluorenylacetamide, 3,3 diphenyl-3-dimethylcarbamoyl-1-propyne, N-methyl-N-nitroso-N'-nitroguanidine, N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide, aflatoxin G1, or ethionine. N-2-Fluorenylacetamide induced hepatocarcinomas more rapidly and in higher incidence in deficient rats than in control rats. 3,3-Diphenyl-3-dimethylcarbamoyl-1-propyneinduced a higher incidence of hepatocarcinomas but not gastric tumors in deficient rats. Aflatoxin B1, included as a positive control, was significantly more hepatocarcinogenic in deficient rats. Gastric tumor induction by N-methyl-N-nitroso-N'-nitroguanidine and induction of tumors of the urinary bladder by N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide were not influenced by diet. Aflatoxin G1 and ethionine were toxic to deficient rats, and carcinogenic doses could not be administered. (Author Abstract)

58. EFFECT OF CYCLOPROPENOID COMPOUNDS ON THE CARCINOGENIC ACTIVITY OF DIETHYLNITROSAMINE AND AFLATOXIN B1 IN RATS.

Nixon JE, Sinnhuber RO, Lee DJ, Landers MK, Harr JR
Dept. Food Sci. Technol., Oregon State U., Corvallis
J Natl Cancer Inst; 53(2):453-458 1974

The cocarcinogenic activity of cyclopropenoid fatty acids (CPFA) was investigated in Wistar and Fischer rats fed food-grade cottonseed oil (0.35% CPFA) or Sterculia foetida oil (50% CPFA) with aflatoxin B1 or diethylnitrosamine (DEN). Wistar rats fed 0.2 or 1.0 mg/kg DEN daily for an average of 7-14 months developed hepatomas with incidence rates of 40 and 70%, resp; they developed no other tumors. Wistar rats fed 20 or 100 ppb aflatoxin B1 showed hepatoma incidence rates of 0 and 43%, resp, and total tumor incidence rates of 10 and 51%, resp. The hepatoma incidence increased from 41 to 61% in male Wistar rats when CPFA was added to the 100 ppb aflatoxin B1 diets, but incidences were only 4 and 14% when CPFA was added to the low and high level DEN diets, resp. Fischer rats fed 0.1 mg/kg DEN were free of other tumors, but 7% developed hepatomas; when CPFA was added to the diet, 10% developed other tumors. The Fischer rats were more sensitive to 20 ppb aflatoxin B1 than the Wistar rats (hepatoma incidence of 21 vs 0%). The incidence of kidney tumors was less than 0.3% in all rats. CPFA exhibited a weak cocarcinogenic effect in specific comparisons, but generally did not influence the carcinogenicity of aflatoxin B1 or DEN.

59. NUTRITIONAL MODULATION OF CARCINOGENESIS (MEETING ABSTRACT).

Newberne PM, Rogers AE, Gross RL
MIT, Cambridge, MA 02139
Third International Symposium On Detection And Prevention Of Cancer; 221 1976

Epidemiologic and laboratory studies have provided evidence that nutrition can modify the response of man and animals to carcinogenesis. Nutrients for which there is strongest association with a modified response include protein, fat, choline-methionine, vitamin A,and riboflavin. This paper presents results with animal models in which the lipotropes (choline-methionine) and vitamin A at marginal concentrations in the diet enhance the induction of colon cancer by dimethylhydrazine. A **diet marginal in vitamin A results in a significant incidence of colon cancer in rats exposed to aflatoxin B1**, an inducer of liver cancer under standard circumstances. Conclusions are that adequate but not excessive intake of lipotropes and of vitamin A result in less susceptibility to environmental carcinogens. (Author Abstract)

60. VITAMIN B12 ACCELERATION OF HEPATOCARCINOGENESIS (MEETING ABSTRACT).

Poirier LA, Wenk ML, Madison RM, Thompson DL, McKay WS
Natl. Cancer Inst., Bethesda, Md.
Proc Am Assoc Cancer Res; 15:51 1974

The carcinogenic activities of 2-acetylaminofluorene (AAF), 4-dimethylaminoazobenzene (DAB), diethylnitrosamine (DEN), and **aflatoxin B1 (Afla) were studied in male weanling rats fed diets that were vitamin B12 - adequate or vitamin B12 -deficient.** AAF, DAB and Afla were administered in the diet at 150, 700 and 3 ppm respectively; DEN was administered in the drinking water at 40 ppm. The vitamin B12 -adequate diet consisted of the vitamin B12 - deficient diet containing 0.1 ppm vitamin B12 . Virtually all animals treated with carcinogens for more than 12 weeks developed hepatomas. However, the mean survival times in days +- S.E. from the start of the experiment for the rats dying with hepatocellular carcinomas were as follows: AAF, 174 +- 13, 240 +- 9; DAB, 207+- 18, 264 +- 19; DEN,98 +- 3, 117 +- 3; and Afla, 197 +- 14, 195 +- 16 in the vitamin B12 -adequate and the vitamin B12 -deficient groups, respectively. The toxicity of the hepatocarcinogens also appeared to be greater in the vitamin B12 -adequate groups than in the vitamin B12 - deficient groups. These results indicate that vitamin B12 may play an influential role in hepatocarcinogenesis.

61. RETINOIDS AND CARCINOGENESIS.

Sporn MB
Lung Cancer Branch, Div. Cancer Cause and Prevention, NCI, Bethesda, MD 20014
Nutr Rev; 35(4):65-69 1977

Discussion is made of the role of vitamin A and its analogs, the retinoids, in facilitating the differentiation of epithelial cells and in carcinogenesis. Retinoid deficiency enhances susceptibility to carcinogenesis by a variety of stimuli, including exposure to polycyclic hydrocarbons, nitrofurans, and aflatoxin B1, in several tissues. These include the respiratory tract, bladder, and colon of the rat and the respiratory tract of man. Pharmacological administration of vitamin A and its synthetic analogs has been used successfully to prevent cancer of the skin, lung, bladder, and breast in experimental animals in which the dietary intake of vitamin A would have been considered adequate for most nutritional purposes. Retinyl methyl ether administered to rats in their diet 1 wk after a single oral dose of 7,12-dimethylbenzanthracene delayed the occurrence and reduced the incidence of subsequent mammary cancer. The inhibitory effects were not the result of inhibition of the initiation of carcinogenesis, but of the progression of premalignant states of the disease to invasive malignancy. The future practical use of these agents for cancer prevention in man will depend upon the availability of appropriate synthetic retinoids. (41 Refs)

62. INHIBITION OF HEPATOCARCINOGENESIS BY ADRENOCORTICOTROPIN IN AFLATOXIN B1-TREATED RATS.

Chedid A, Bundeally AE, Mendenhall CL
Department of Pathology, University of Cincinnati, College of Medicine, 231 Bethesda Ave., Cincinnati, Ohio 45267
J Natl Cancer Inst; 58(2):339-349 1977

We examined whether hormones would modify the carcinogenic action of aflatoxin B1 (AFB1). Four groups of inbred Fischer rats received AFB1, 125 ug per animal, weekly per os. In three of the groups, certain hormones were administered simultaneously: One group received 1 U growth hormone (GH) sc weekly, another was given 4 U adrenocorticotropin (ACTH) weekly, and a third received 0.5 U insulin weekly sc. AFB1, ACTH, and insulin were given for 20 weeks; GH was given for only 10 weeks. The control group did not receive hormone adjuvant. In each group, 4 animals were killed at 7, 14, 21, 28, and 35 weeks; the remaining rats were killed at 77 weeks. Their livers were carefully examined and samples prepared for light and electron microscopy. Animals receiving AFB1 and ACTH failed to exhibit hepatocellular carcinoma. On the other hand, malignant lymphoma appeared at 56 weeks in 3 of the 6 surviving males on this regime. AFB1, alone or when given with insulin or GH caused hepatocellular carcinoma in all animals; in these, lymphoma was not observed. Lymphoma comprised two cell types, each with similar nuclear characteristics but differing in their nucleocytoplasmic ratios and in the amount and distribution of cytoplasmic organelles. Alterations leading to hepatocellular carcinoma were examined at various stages of development. 'Basophilic hyperplasia' reflected an increase in free ribosomes. 'Hyperplastic nodules' were composed of hepatocyte aggregates with characteristics similar to those encountered in the earlier stage. Both the 'neoplastic nodules' and hepatocellular carcinomas were formed by cells containing large, 'smooth fingerprints' and free ribosomal aggregates. These features supported the concept that AFB1 impairs ribosomal binding to endoplasmic reticulum membranes. The failure of ACTH-treated animals to develop hepatocellular carcinoma was ascribed to the effect of adrenal cortical stimulation upon membrane-polysome binding. (Author Abstract)

63. SOME INTERACTIONS OF LIGHT, RIBOFLAVIN, AND AFLATOXIN B1 IN VIVO AND IN VITRO.

Joseph-Bravo PI, Findley M, Newberne PM
Dept. Nutrition and Food Science, Massachusetts Inst. Technology, Cambridge, MA
J Toxicol Environ Health; 1(3):353-376 1976

The interaction of riboflavin, aflatoxin, and light was studied with Sprague-Dawley rats. In a study of carcinogenesis rats received 25 ug aflatoxin B1 (in 0.2 ml dimethylsulfoxide (DSMO) intragastrically) 5 days/wk for 3 wk; 30 min after each dosing, half of them were irradiated for 2 hr. Glucose-6-phosphatase levels 5 days after treatment were about twice as high in livers from aflatoxin-treated (with or without light treatment) as in livers from DSMO-treated rats. At 53 wk, all 11 of the aflatoxin-treated rats remaining had liver parenchymal tumors; only 5 of 12 of the aflatoxin plus light-treated rats had such tumors. In the second experiment, rats were dosed with riboflavin (1 or 10 mg/kg) and divided into four groups: no further treatment; aflatoxin (LD50); irradiation (1-2 hr); or aflatoxin plus irradiation. Aflatoxin caused blood riboflavin levels to decrease. This effect was enhanced by irradiation, suggesting that photosensitized riboflavin and aflatoxin form a complex. Studies in vitro showed that riboflavin quenched aflatoxin photodegradation, perhaps by complexing with aflatoxin. Thus, low, carcinogenic doses of aflatoxin may complex with endogenous, photosensitized riboflavin, inhibiting its degradation into carcinogenic metabolites.

64. STIMULATION OF GROWTH OF CARCINOGEN-INDUCED MAMMARY CANCERS IN RATS BY THYROTROPIN-RELEASING HORMONE.

Chen HJ, Bradley CJ, Meites J
Department of Physiology, Neuroendocrine Research Laboratory, Michigan State University, East Lansing, MI 48-824
Cancer Res; 37(1):64-66 1977

Twice-daily injections of three different doses of synthetic thyrotropin-releasing hormone (TRH), a hormone normally produced by the hypothalamus, produced significant increases in size and number of 7,12-dimethylbenz(a)anthracene-induced mammary cancers over 0.87% NaCl solution-injected control rats. When thyroidectomized rats, bearing 7,12-dimethylbenz(a)anthracene-induced mammary tumors were given the same twice-daily injections of TRH, mammary tumor growth was increased to the same extent as in intact rats given TRH, showing that the effects of TRH were not exerted via stimulation of thyroid function. The TRH-induced increments in mammary tumor growth were accompanied by significant increases in serum prolactin levels over 0.87% NaCl solution-injected controls. A single daily injection of 2-bromo-alpha-ergocryptine (CB-154), a prolactin-release inhibitor, completely blocked TRH-induced mammary tumor growth and reduced serum prolactin values. These results indicate that a twice-daily pulse of TRH can stimulate mammary tumor growth by releasing prolactin from the anterior pituitary. (Author Abstract)

65. HORMONAL INFLUENCES ON RNA-POLYMERASES DURING CARCINOGENESIS (MEETING ABSTRACT).

Chedid A, Bundeally A
Dept of Pathology, Chicago Med School, Chicago, Ill 60612
Fed Proc; 36(3):1078 1977

The purpose of this work was to assess the role of adrenocorticotrophin (ACTH), insulin and growth hormone (GH) in the initiation and regulation of neoplastic growth and to study their influence on RNA polymerases during tumorigenesis using aflatoxin B1 (AFB1) hepatocarcinoma as a model. Fisher 344 rats were treated orally with AFB1 (125 mug) daily for five days per week. They were divided into four groups, three of these groups received simultaneously hormones, for group 1, ACTH (4U); group 2, insulin (0.5U); group 3, GH (1U); group 4, AFB1 alone. Another group served as untreated controls. AFB1, ACTH and insulin were given for 20 weeks and GH for 10 weeks. Four animals per group were sacrificed on weeks 7, 14, 21, 28, 35 and 77 and their livers assayed for RNA polymerases. AFB1 treatment alone induced hepatocarcinoma in all animals, noticed first at the 35th week after the initial exposure. Animals receiving AFB1 and ACTH did not develop hepatocarcinoma even after 77 weeks while those receiving insulin had small tumors. RNA polymerase I was increased in the animals receiving ACTH or insulin for as long as treatment lasted. There were no changes in RNA polymerase II or III. The relationship between ACTH protection and elevated RNA polymerase I is unclear. (Author Abstract)

66. DRUG METABOLISM AND AFLATOXIN-INDUCED HEPATOMA IN RAINBOW TROUT (SALMO GAIRDNERI).

Scarpelli DG
Univ. Kansas Medical Center, Coll. Health Sciences and Hosp., Kansas City, KS 66103
Prog Exp Tumor Res; 20:339-350 1976

The effects of aflatoxin B1 and b-diethylaminoethyldiphenyl propylacetate hydrochloride (SKF-525A) on drug metabolism by liver microsomes in rainbow trout (Salmo gairdneri) and the modification of aflatoxin B1 hepatocarcinogenesis by chronic inhibition of drug metabolism with SKF-525A were investigated. Two ip injections of 0.05 mg/kg aflatoxin B1 during 1 wk resulted in a significant increase in the activity of the drug-metabolizing enzyme system; administration of SKF-525A (5 mg/kg) prior to aflatoxin B1 inhibited activation of the system. The two injections of aflatoxin B1 led to a proliferation of smooth-surfaced endoplasmic reticulum (ER) to the extent that ER patches were present in cytoplasmic areas far from the foci of glycogen accumulation. Other organelles showed no consistent alterations in morphology or number. Hepatocytes of trout injected with SKF-525A prior to aflatoxin B1 did not contain cytoplasmic accumulations of smooth-surfaced ER. Fifty injections of a subnecrotic dose of aflatoxin B1 induced grossly evident hepatomas in 16/20 fish. These were histologically diagnosed as trabecular hepatomas. The livers of three fish that did not show gross evidence of tumor nodule formation had numerous foci of proliferating basophilic hepatocytes. In one fish, no gross or microscopic lesions were present. In the 17 fish that survived 50 injections of SKF-525A followed by aflatoxin B1 injections, only one developed a grossly evident hepatoma, and only two livers showed microscopic proliferative basophilic lesions. The remaining 14 fish were free of both gross and microscopic evidence of hepatoma formation. These results are in agreement with those obtained in higher homeothermic vertebrates and illustrate the apparent unity of basic organization and function that exists in phylogenetically diverse biological systems. (35 refs)

D. Modification of Biochemical or Toxicological Actions of/by Mycotoxins

67. PROTECTIVE EFFECT OF BENZOFLAVONE AND ESTROGEN AGAINST 7,12-DIMETHYLBENZ(A)ANTHRACENE- AND AFLATOXIN-INDUCED CYTOTOXICITY IN CULTURED LIVER CELLS.

Schwartz AG
Temple U. Med. Sch., Philadelphia, Pa.
Cancer Res; 34(1):10-15 1974

Cultured liver epithelial cells (E-3) established from an adult male Sprague-Dawley rat were incubated in media containing 7,12-dimethylbenz(a)anthracene (DMBA) with or without 7,8-benzoflavone or a steroid. After 24 hr in these media and 24 hr in normal medium, 3H-thymidine was added to the cultures. 7,8-Benzoflavone exerted a protective effect on the DMBA-induced inhibition of acid-insoluble 3H-thymidine incorporation by the liver cells; the protective effect was dependent on the concentration of 7,8-benzoflavone. 7,8-Benzoflavone also exerted a protective effect against the depression in acid-insoluble 3H-thymidine incorporation produced by treatment of the E-3 cells with either aflatoxin B1 or sterigmatocystin. Estrogenic steroids, which have previously been shown to protect against the cytotoxic effect of DMBA, also protected against the cytotoxic effect of aflatoxin B1. These observations may be related to the high incidence of primary liver cancer in areas of Africa and Southeast Asia and to the increase in cancer incidence with age. The decline in plasma estrogen levels in women with age may be involved in cancer development.

68. MYCOTOXINS, BIOELEMENTS AND PERSPECTIVES OF ECOLOGICAL PROPHYLAXIS OF LEUKEMIA.

Aleksandrowicz J
Clinica Hematologica, IV Clinica Medica, Universidad de Cracovia, Krakow, Poland
Rev Esp Oncol; 22(2):311-334 1975

An ecological study correlating physical and chemical agents, viruses and the body's immunological condition with the appearance of leukemias was performed. Leukemia is a manifestation of adaptation to a disturbed balance between the activity of antioxidants and leukemogenic agents, giving rise to a deficient immunological response. Antioxidants, such as vitamin C and E, Mg+1, Se+1 and other solid oligoelements, diminish because of the excessive refining of food and the artificial fertilization of the soil. This lessening produces immunological alterations by thymus damage, which facilitates the development of proliferative process of the lymphoreticular system after the action of aflatoxins and viruses. Sodium selenate inhibits the toxic effects of aflatoxin B1 by protecting the activity of the thymus and lymphocytes and by stimulating the synthesis of antibodies. (36 refs)

69. INFLUENCE OF DDT PRETREATMENT OF A DUCKLING ON ITS SUBSEQUENT REACTION TO PEANUT OIL CAKE INFECTED WITH ASPERGILLUS FLAVUS.

Ferrando R, N'Diaye A, Henry N, Fourlon C, Gautier F
Natl. Vet. Sch., Alfort, France
C R Acad Sci D (Paris); 279:1131-1134 1974

Male ducklings aged one day (type unspecified) were given a diet of 60% barley flour and 25% either plain soybean oil cake or 25% peanut oil cake infected with 0.9 mg/kg Aspergillus flavus. Groups of ducklings receiving the aflatoxin diet received in addition 150 mg/kg DDT or 100 mg/kg phenobarbital. One group receiving the DDT supplement was given a diet of soy for eight days, then the infected peanut diet. For every type of diet there were subgroups receiving either 10,000 or 30,000 IU/kg vitamin A. Average weight gain over a period of four weeks and an index of food consumption were calculated for each group of ducklings. The vitamin A supplement doses produced no significant differences in the results. Phenobarbital supplement produced a significant improvement in both the weight gain and the index of food consumption compared with the groups given aflatoxins alone: ducklings given aflatoxins and phenobarbital gained 667.5 g and 654.9 g compared with 472.9 and 452.1 g for the ducklings given aflatoxins without phenobarbital; and the food consumption indices of the first groups were 2.03 and 1.90 compared with 2.30 and 2.41 for the ducklings given aflatoxins alone. The ducklings given DDT supplements gained 523.4 g and 541.0 g, and the food consumption indices were 2.20 and 2.53. The groups of ducklings treated with DDT for eight days before being given aflatoxins gained 697.4 g and 673.9 g. The food consumption indices were 2.18 and 2.16. All ducklings that were fed aflatoxins showed an increase in the ratio of liver weight to body weight, ranging from .0479 to .0628, compared with that of the control ducklings, ranging from .0386 to .9359. The partial detoxification of the aflatoxins by phenobarbital and DDT is thought to result from induction of cytochrome P 450 formation by the microsomes and perhaps by inhibition of hemiacetal aflatoxin formation.

70. INDUCTION OF MIXED FUNCTION OXIDASES ON ORAL ADMINISTRATION OF DIELDRIN.

Kohli KK, Maggon KK, Venkitasubramanian TA
Dept. Biochemistry, Vallabhbhai Patel Chest Inst., Univ. Delhi, Delhi-1 10007, India
Chem Biol Interact; 17(3):249-255 1977

The effect of dieldrin on the metabolism of aflatoxins B1 and G1 by male Wistar rats was investigated in liver microsomal preparations from treated animals. The metabolism of the aflatoxins was increased, indicating an increased mixed function hydroxylase activity. This could be due to an increase in protein synthesis. (37 Refs)

71. DIETARY FACTORS WHICH MODIFY AFLATOXIN TOXICITY IN THE RAT.

Wells PA
U. California, Los Angeles
Diss Abstr Int B; 34(11):5538-B 1974

Male weanling rats were maintained on diets containing 1.7 ppm aflatoxin and: no fat; saturated fat; polyunsaturated fat; or polyunsaturated fat in conjunction with low, normal or high levels of dietary protein. Aflatoxin inhibited growth in all groups except those maintained on low protein diets. Liver size varied as a function of the severity of liver tumors and was most increased in animals on normal and high-protein diets. The aflatoxin-induced increase in liver size was small in animals maintained on saturated fat diets. Liver size was not increased in rats fed low protein diets or low protein diets supplemented with cystine. Liver cholesterol levels were increased in all animals except those fed low protein diets. The elevation was greatest among those fed saturated fats; sterculic acid markedly diminished this effect. In the sterol ester and triglyceride fractions of the plasma, the C18:2/C20:4 ratio increased with a decrease in the incidence and severity of tumors. C16:0 and C18:0 decreased and C18:1 increased in the liver sterol fraction in response to aflatoxin, except in the low protein group. Aflatoxin did not produce changes in the liver triglyceride fraction, which was, however, altered in the low protein group. C18:0 was decreased and C18:1 increased in the liver phospholipid fraction in response to aflatoxin, except in the low protein group. Thus, low protein diets, and to a lesser extent saturated fat-containing diets, protect against aflatoxin toxicity.

72. CERTAIN GENERAL ASPECTS OF THE PROBLEM OF AFLATOXINS.

Ferrando R
Service de Nutrition et d'Alimentation, Ecole Nationale Veterinaire, 94701 Alfort, France
Med Chir Dig; 2:59-60 1975

The question of aflatoxins is a vast and far-ranging one. It requires basic research from several angles: human and animal pathology, and evolution and prevention of mycotoxicosis. **Aflatoxins cause a faster depletion of the hepatic reserve of vitamin A (retinol).** The decrease of retinol in the liver is accompanied by a significant reduction of the zinc supply. In the absence of zinc, the action of vitamin A is impaired. Lastly, the lack of vitamin D3 intensifies the damages caused by aflatoxins. This has just been demonstrated in ducklings. A deficiency in vitamin D3 retarded their growth in the presence of aflatoxin, with a weight gain of 174 g in 22 days against a corresponding increase of 415 g in a control group. With a dose of 750 IU/vitamin D3 /kg of feed, the weight gains were, respectively, 301 and 529 g. Ossification troubles seemed more frequent among the subjects receiving aflatoxins. Thus, nutrition and toxicology meet repeatedly on the subject of aflatoxins.

73. MICROSOMAL METABOLISM AS A DETERMINANT OF AFLATOXIN TOXICITY.

Tilak TB, Nagarajan V, Tulpule PG
Natl. Inst. Nutrition, Indian Council Medical Res., Hyderabad 500007 India
Experientia; 31(8):953-954 1975

Evidence that suggests that the metabolism of aflatoxin produces compounds more effective in hepatocarcinogenesis but less effective in producing acute lesions is reviewed. For example, protein or vitamin A deficiency increases the acute symptoms but reduces the incidence of hepatomas.

74. DEVELOPMENT OF RESISTANCE TO CYTOTOXICITY DURING AFLATOXIN CARCINOGENESIS.

Judah DJ, Legg RF, Neal GE
MRC Toxicology Unit, Woodmansterne Rd., Carshalton, Surrey SM5 4EF, England
Nature (Lond); 265(5592):343-345 1977

The relationship between the development of resistance to cytotoxicity and carcinogenesis was investigated using the mycotoxin aflatoxin B1. Feeding young adult male Fischer rats a diet containing a low concentration (4-5 ppm) of aflatoxin B1 for 6 wk, followed by return to the control diet, resulted in a 100% incidence of hepatocarcinoma. During the first 3 wk, histological examination of the liver indicated an acutely toxic response that resulted in the death of a considerable proportion of the hepatocytes at the end of the third week. The second 3-wk period, during which the carcinogenic response was initiated, was accompanied by a proliferation of parenchymal and nonparenchymal cells and recovery of nucleic acid synthesis. This indicates that these cells have a mechanism that makes them resistant to the acutely toxic but not the carcinogenic actions of aflatoxin. The resistance of the liver cells to the toxic action of aflatoxin B1 was also examined in vitro. Hepatocytes were isolated and subsequently cultured from livers of control animals (BL8) and livers of animals that had received the aflatoxin-contaminated diet (BL7) for 6 wk. The cells appeared morphologically similar, but when they were treated with aflatoxin, the BL7 cells survived but the BL8 cells became structurally disorganized and detached from the dish. Rapidly dividing epithelial cell lines were established from each of the original maintenance cultures, and the sensitivity of the cells to aflatoxin B1 was again determined after several passages. Resistance of the original hepatocytes in the case of the aflatoxin-fed rats was still present in the cell lines derived from them. These results demonstrate that the resistance to the acutely toxic action of aflatoxin B1, once established in the cells, is stable. (9 Refs)

75. INTERACTION OF AFLATOXICOSIS WITH HEAT STRESS.

Wyatt RD, Thaxton P, Hamilton PB
Dep. Poult. Sci., North Carolina State Univ., Raleigh
Poult Sci; 54(4):1065-1070 1975

The effects of high temperature stress on cloacal temperature, body fat, and serum glucose in aflatoxin-treated chickens were investigated. Aflatoxicosis was induced in 1-day-old males by incorporating various doses of aflatoxin (0, 0.625, 1.25, 2.5, 5.0 and 10.1 microg/g) in a diet from which all medications had been omitted. Four groups of ten birds were fed each diet for three weeks. The mean survival times of 3-week-old chickens fed the aflatoxin-containing diets since hatching were determined at 37 C and 45% relative humidity and at 40 C and 45% humidity. Both serum glucose and total body fat were decreased significantly by doses greater than or equal to 2.5 microg/g. Cloacal temperature was decreased slightly but significantly in chickens fed 5 or 10 microg/g for 12 days or longer. The mean survival time of birds exposed to heat stress of 40 C and 45% relative humidity did not vary with the dose of aflatoxin. A milder stress of 37 C and 45% relative humidity caused chickens fed aflatoxin at doses greater than or equal to 2.5 microg/g to show decreased survival times in comparison to the controls. These data might be explained by assuming that the lessened burden of body fat during aflatoxicosis accounts for the increased survival time in a severe heat stress (43 C and 45% relative humidity), but that other parameters related to physiological stress play a dominant role during a less severe but more prolonged heating episode. It is also suggested that the hypoglycemia, hypothermia, and lessened body fat account for the previously reported increased sensitivity to a lethal cold exposure during aflatoxicosis.

76. THE EFFECT OF SODIUM SELENATE ON CYTOTOXIC ACTION OF AFLATOXIN B1 IN REGARD TO CULTURED HUMAN LYMPHOCYTES AND TO EMBRYONIC DEVELOPMENT OF XENOPUS LAEVIS.

Aleksandrowicz J, Dobrowolski J, Lisiewicz J, Smyk B, Skotnicki AB
Hematological Clinic, Inst. Internal Medicine, Medical Acad. Cracow, Poland
Rev Esp Oncol; 23(1):7-11 1976

The effect of the addition of sodium selenate on phytohemagglutinin-treated cultures of lymphocytes intoxicated with aflatoxin B1 was determined. Selenate produced a moderate increase in the number of transformed cells and in the mitotic index. Concentrations above 1.0 microgram/ml were toxic to the lymphocytes. Aflatoxin decreased the number of mitoses and the number of transformed cells. Selenate produced a full recovery of the lymphocyte response to phytohemagglutinin. Aflatoxin and selenate were also added to an aqueous solution containing normal Xenopus laevis embryos at different stages of early embryonal development. Compared with controls, aflatoxin (less than 2.5 microg/ml) did not change the viability of the embryos after a 3-day treatment. The addition of selenate to the medium at the start of cleavage resulted in a significant reduction of lethality due to aflatoxin. The antioxidant properties of selenium may restore the immunological reactivity of lymphocytes damaged by aflatoxin. Furthermore, the protective effect of selenium with regard to the influence of aflatoxin on embryonic development of Xenopus laevis and on lymphocyte transformation may be related to its stabilizing effect on nucleic acids. (9 refs)

E. Histological and/or Morphological Changes Resulting from Mycotoxin Exposure In Vivo

77. EARLY CELL CHANGES IN THE COURSE OF CHEMICAL CARCINOGENESIS. (PP. 89-102)

Butler WH
Fundamentals in Cancer Prevention, Proceedings of the 6th International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, 1975. Magee PN, Takayama S, Sugimura T, Matsushima T, ed. Baltimore, Univ Park Press, 1976.

The structural and biochemical properties of cells undergoing chemically induced neoplastic changes are reviewed. The sequential effect of an LD50 dose of dimethylnitrosamine (DMN) on renal epithelial and mesenchymal cells in survivors was studied. Unequivocal evidence of autonomous growth and invasion required about 20 wk. Continuous cultures of kidney cells obtained 20 hr after in vivo treatment with DMN exhibited properties similar to those of transformed cells. Thus, neoplasia can be induced as an acute response and only time is required for a full demonstration of autonomous growth, invasion, and metastases in the host. Examination of the liver of inbred male Fischer rats fed 5 ppm aflatoxin for 6 wk revealed foci composed of either small basophilic or large eosinophilic parenchymal cells. Some basophilic foci contained starvation-resistant glycogen and/or a reduced amount of glucose-6-phosphatase; both foci showed a reduction of succinic dehydrogenase and aniline hydroxylase and a patchy loss of membrane ATPase. During the 6-wk feeding period, normal maturation to tetraploid parenchymal cells was reduced, resulting in a shift to a diploid population that persisted for the life of the animal. A major problem in interpreting these findings is which, if any, of the observed foci represent developing neoplasia and which represent a toxic response. (12 Refs)

78. EARLY CYTOLOGICAL CHANGES INDUCED IN RAT LIVER CELLS BY CHEMICALS. (PP. 103-111)

Masahito P, Takayama S, Yamada K
Fundamentals in Cancer Prevention, Proceedings of the 6th International Symposium of The Princess Takamatsu Cancer Research Fund, Tokyo, 1975. Magee PN, Takayama S, Sugimura T, Matsushima T, ed. Baltimore, Univ Park Press, 1976.

Cytogenetic studies on the effect of seven hepatocarcinogens (4-dimethylaminoazobenzene, DAB; 3'-methyl-DAB; 2-acetylaminofluorene, 2-AAF; 2,7-AAF; aflatoxin B1; alpha-benzene hexachloride; diethylnitrosamine) and seven nonhepatocarcinogens (N-nitrosobutylurea; 2-Me-DAB; methionine; maleic hydrazide; DDT; furylfuramide cigarette tar) on rat liver mitosis are reviewed. Male Donryu rats (8 wk old, 150 g) were given the compounds in the diet or drinking water for 3 wk. They were killed 3, 7, and 11 wk after the start of the treatment, and the liver tissue was examined microscopically. Mitotic rates in the livers of rats treated with the hepatocarcinogens were 10 times higher than those in controls (0.02%) after 3 wk, but they decreased to 0.05% after 11 wk. Except for N-nitrosobutylurea, which caused a slight increase, the nonhepatocarcinogens had no effect on mitotis. In rats sacrificed shortly after hepatocarcinogen treatment, (other than with alpha-BHC) over 95% of the dividing cells were diploid, the remainder being tetraploid; in the alpha-BHC rats, 30% of these cells were tetraploid. Nonhepatocarcinogens generally increased the proportion of tetraploid cells. However, after 11 wk, the frequency of diploid cells was about 90%, regardless of the compound administered. Chromosome aberrations observed in liver cells in rats treated with hepatocarcinogens were mostly chromatid breaks and monocentric markers due to translocation between two chromosomes. In rats treated with nonhepatocarcinogens, mostly chromatid breaks occurred. These increased mitotic rates, changes in ploidy rates, and chromosome abnormalities might be useful in screening for hepatocarcinogens. (5 Refs)

79. INDUCTION OF MICRONUCLEI IN MOUSE AND HAMSTER BONE-MARROW BY CHEMICAL CARCINOGENS.

Friedman MA, Staub J
Dept. Pharmacology, Medical Coll. Virginia, Health Sciences Div., Virginia Commonwealth Univ., Richmond, VA 23298
Mutat Res; 43(2):255-261 1977

Four carcinogens, dimethylnitrosamine (DMN), 3-methylcholanthrene (3-MC), acetylaminofluorene (AAF), and aflatoxin B1 (AFB1) were tested in the micronucleus test using mice (acute tests) and hamsters (acute and subacute tests). Male and female Swiss mice (DUB:ICR) and male Golden Syrian hamsters were used. The compounds were administered ip 30 and 6 hr prior to killing (in the acute tests). In the subacute tests, the compounds were administered three times per wk for 1 to 12 wk. Animals injected with triethylenemelamine were used as controls. 3-MC induced a dose-dependent increase in the number of micronuclei, yielding a 2.65-fold increase over controls at a dose of 60 mg/kg. At 1,000 and 2,000 mg/kg AAF, the incidence of micronuclei showed 3.0- and 5.04-fold increases, respectively. The RBC to nucleated cell ratio was 38.9% at 1,000 mg/kg AAF and 53.7% at 2,000 mg/kg. At 15 and 20 mg/kg, AFB1 increased the incidence of micronuclei by 1.65- and 2.67-fold, respectively. No bone marrow toxicity was observed with AFB1. With DMN, considerable bone marrow toxicity was seen beginning at 14 mg/kg, and mortality was seen at 50 mg/kg. At 2 mg/kg DMN, there was a 1.48-fold increase in the number of micronuclei; this reached a maximum of 2.89-fold at 14 mg/kg. In hamsters, all of the test compounds except AFB1 increased the percentage of micronuclei. The data indicate that the environmental carcinogens tested were all active in the micronucleus test. Therefore, marrow toxicity must be considered during evaluations of the mutagenic activity of carcinogens with the micronucleus test. The micronucleus test did not appear useful in chronic protocols, but the acute studies showed that this procedure is of value in the characterization of the mutagenic potential of a compound. (7 Refs)

80. EXPERIMENTAL HEPATOCELLULAR CARCINOGENESIS.

Newberne PM

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Cancer Res; 36(7):2573-2578 1976

The sequential development of lesions in the liver leading to hepatocellular carcinoma in experimental animals appears to follow a similar pattern irrespective of the carcinogen. The speed with which the lesions develop is related to the chemical, the dose and dosing schedule, and the diet fed. In studies with aflatoxin B1 there is a predictable series of morphological expressions beginning with focal areas of hydropic degeneration (Stage 1); hyperplastic basophilic cells, singly or in close association with Stage 1 (Stage 2); nodular hyperplasia of parenchymal cells which become progressively more abnormal (Stage 3); transitional cell changes (Stage 4); and, ultimately, hepatocellular carcinoma (Stage 5). (Author Abstract)

81. ON THE PATHOGENESIS OF EXPERIMENTAL HEPATOCELLULAR CARCINOMA. (PP. 3-22)

Farber E

Hepatocellular Carcinoma. Okuda K, Peters RL, ed. New York, John Wiley and Sons Inc, 1976.

The pathogenesis of experimental hepatocellular carcinoma is examined. The compounds currently considered the most significant in relation to human liver cancer are the mycotoxins, especially the aflatoxins. Most hepatocarcinogens are not carcinogens per se, but are metabolically converted to highly reactive derivatives. These activated electrophiles undergo a variety of reactions with components in nucleic acids, proteins, and probably many other cellular constituents. Biologically, the initiation of hepatocellular carcinoma seems to consist of the induction of a new hepatocyte population that differs in several respects from the original. It is more resistant to the cytotoxic environment created by the carcinogenic process, which allows it to proliferate. It also contains an antigen so far not found in normal liver, and it proliferates in patterns resembling fetal and neonatal liver. Early in the development of liver cancer, the carcinogen induces a new cell population that appears as multiple scattered foci, creates an appropriate selection pressure, and interrupts differentiation or remodeling. The phenomenon of arrested differentiation or aborted development may be an important principle in the pathogenesis of hepatocellular carcinoma. Both somatic mutation and altered differentiation may each play a significant role at different stages of the process. There appears to be a continual selection of smaller and smaller numbers of cells, each with an increasingly greater probability of development of cancer. (123 Refs)

82. ULTRASTRUCTURAL AND AUTORADIOGRAPHIC STUDIES OF THE EFFECT OF AFLATOXIN G1 ON HEPATIC CELLS.

Kim CS, Lee YB

Dept. Pathology, Yonsei Univ. Coll. Medicine, Seoul, Korea

Yonsei Med J; 15(1):17-26 1974

To investigate the toxicity of aflatoxin G1 and its mechanism of action, light microscopic, histochemical, electron microscopic, and autoradiographic studies were made on rat liver at various time intervals after administration of 3 mg of the drug ip. Light microscopic alteration was first observed at 6 hr, and necrosis of periportal hepatic cells was found at 18 hr. However, reduction of Feulgen positivity of the nucleus and pyroninophilia of cytoplasm were observed as early as 1 hr. Ultrastructural changes were noted at 6 hr and were advanced at 18 hr. Early changes consisted of nucleolar segregation, dilatation of rough endoplasmic reticulum, swelling of mitochondria, and detachment of membrane bound ribosomes, followed later by disruption of cytoplasmic organelles and focal necrosis. These changes were most marked at the periportal region. Autoradiography showed inhibition of H^3-uridine incorporation into the nucleus at 1 hr, which was most marked at 6 hr and then showed some recovery at 18 hr. H^3-uridine labeling in the cytoplasm was also inhibited, and the most marked inhibition was noted at 1 hr after aflatoxin administration. These data indicate that aflatoxin G1 has a hepatotoxic effect, particularly at the periportal region, and probably due to inhibition of nuclear RNA synthesis, which leads to inhibition of ribosomal RNA and eventually protein synthesis. The DNA synthesis is also inhibited, as shown by reduction of the Feulgen reaction in the nucleus. (24 refs)

83. FINE STRUCTURE OF PRENEOPLASTIC CELLS IN THE LIVER (MEETING ABSTRACT).

Zaki FG, Greenlee JA, Keysser CH

Pathology Dept., Squibb Inst. Medical Res., New Brunswick, NJ 08903

Lab Invest; 32(3):441-442 1975

The hepatocarcinogenicity of diethylnitrosamine, dimethylaminoazobenzene, 2-acethylaminofluorene, and aflatoxin B is well documented in mice and rats. Several wk before the development of the hepatomas, the livers of animals dosed with these carcinogens have hepatocytes entirely devoid of glucose-6-phosphatase activity. These transformed hepatocytes, which appear first singly and then in groups, retain their structure in hematoxylin and eosin-stained sections. These preneoplastic cells have already aquired some of the ultrastructural features of hepatoma cells such as dilation and disorganization of the endoplasmic reticulum, dispersal of ribosomes, depletion of glycogen, enlargement of the Golgi vesicles, and increased number of lysosomes and pinocytotic vesicles. Morphologic changes indicative of neoplastic tranformation involve the surface membranes of these cells and include marked loss of sinusoidal microvilli protrusion of cytoplasmic blebs in the intercellular spaces, microvillization and folding of the apposing cell membranes, reduction of desmosomes and tight junctions, and formation of blebs clogging the bile canaliculi. Nuclear manifestation of neoplastic transformation appears in the form of nucleolar segregation or capping. (no refs)

84. HISTOLOGIC CHANGES IN LIVERS OF PYRIDOXINE-DEPRIVED BABOONS-RELATION TO ALPHAI FETOPROTEIN AND LIVER CANCER IN AFRICA.

Foy H, Kondi A, Davies JN, Anderson B, Parker A, Preston J, Peers FG
Wellcome Trust Res. Lab., Nairobi, Kenya
J Natl Cancer Inst; 53(5):1295-1311 1974

Histological changes occurring in the liver of 15 baboons fed a pyridoxine-deficient diet were observed. The purpose of the study was to assess any relation between these changes and the presence of alpha1 fetoprotein (AFP) and their connection with the high incidence of primary liver cancer in humans in certain parts of Africa. Changes due to pyridoxine deprivation were compared with liver histologic changes in a second group of 15 animals fed the known carcinogen aflatoxin B1. Ten baboons were intermittently fed a balanced artificial diet lacking only pyridoxine for 2 to 6 yr. Half developed increased amounts of AFP in their sera and had multiple atypical hyperplastic nodules regarded in some as neoplastic or premalignant. The animals had megalocytosis and anisocytosis, multinucleosis, abnormal mitoses, liver cell plates more than 2 cells thick, and sheets of bile ductule cells; the combination suggests neoplasia. Five other baboons totally deprived of pyridoxine died within 6 to 8 months, with rapidly developing hepatocellular injury. None had atypical hyperplastic nodules, abnormal liver cell plates, increased bile ductule cells, AFP, or signs of tumor, probably because of early death. Fifteen other baboons were given aflatoxin B1, accompanied, preceded, or followed by pyridoxine deprivation. Their liver changes were not as severe as those seen in the animals given the pyridoxine diet alone. This may be attributed to the stimulation of some immunologic mechanism. In all animals deprived of pyridoxine, the serum B6 levels fell from a normal range of 200-350 ng/ml to 5-15 ng/ml, and increased tryptophan metabolites appeared in the urine. The fact that neither atypical hyperplastic nodules nor AFP was present in half the baboons, regardless of these low serum B6 levels, suggests that pyridoxine deficiency does not produce its effects directly, and is possibly associated with differences in immunologic competence.

85. HISTOCHEMICAL MANIFESTATIONS OF EARLY AND LATE CHANGES INDUCED BY AFLATOXIN IN RATS.

Dutu R
Dept. Morphology, Oncological Inst., Bd. 1 Mai, 11, Bucharest, Romania
Acta Histochem (Jena); 57(1):34-43 1976

The histochemical manifestations of late and early changes induced by aflatoxin B1 (5 and 7 mg/kg, ip) in Wistar rats were studied. In the first group (5 mg/kg), there was an alteration of the liver carbohydrate metabolism, as shown by the reduction of polysaccharides content (acid and neutral mucopolysaccharides) and lactic dehydrogenase activity; a slight change in the Krebs cycle as shown by a reduction in NADH2-tetrazolium reductase activity; and a change in lipid metabolism. In the second group (7 mg/kg), there was again an alteration of the liver carbohydrate metabolism marked by a decrease of neutral and acid mucopolysaccharides, predominantly in the central lobe, a decrease of lactic dehydrogenase and ethanol dehydrogenase activities, and a modification of pentose shunt metabolism; these were accompanied by a modification of the Krebs cycle enzymes, protein metabolism, and lipid metabolism. The liver lesions after 86 and 96 wk consisted of large areas of parenchymal cells containing large droplets of fat, with loss of acid and neutral mucopolysaccharides. Particular liver parenchyma alterations were evident in animals reinjected with aflatoxin 92 wk after the first administration. They were characterized by nodules developing degenerative cytoplasmic changes. There was a tendency for regenerating areas to arise at the periphery of these nodules in relation to scattered regions of fibrosis. In the lung and intestine, there were no histological, histochemical, or histoenzymological alterations. The mesenteric lymph nodes of animals from the first group showed a decreased cortical area, the appearance of tingible bodies in germinal centers (day 7), and dilation of the subcapsular and medullary sinuses. The alterations in animals from the second group consisted of blast cells in the paracortical area (day 5), hyperactivity of the paracortical area (day 8), and a decreased cortical area. The results suggest that liver changes are dependent on dose and duration of exposure and may be detected histochemically before significant morphological changes appear. (21 Refs)

86. HISTOCHEMISTRY OF GAMMA-GLUTAMYL TRANSPEPTIDASE IN RAT LIVER DURING AFLATOXIN B1-INDUCED CARCINOGENESIS.

Kalengayi MM, Ronchi G, Desmet VJ
Laboratorium voor Histochemie, Department Medische Navorsing, Fakulteit Geneeskunde, Katholieke Universiteit Leuven, 12 Minderbroedersstraat, B-3000Leuven, Belgium
J Natl Cancer Inst; 55(3):579-588 1975

The histochemical changes of gamma-glutamyl transpeptidase (gamma-GTase) in the livers of rats treated with aflatoxin B1 (AFB1) were studied. Thirty-six male Wistar rats were given, by gastric intubation, small doses of aflatoxin B1 (AFB1): 50 microg twice weekly for four weeks and then 75 microg twice weekly for ten weeks. Their livers were examined histologically and histochemically for gamma-GTase. Before 15 wk no significant hepatic lesions and no changes in gamma GTase activity were detected. From 15 wk hyperplastic liver cell foci appeared. Composed of either clear vacuolated, hyperbasophilic, or eosinophilic liver cells, they progressively increased in number and size. Regardless of their morphologic differences, they all showed a strong gamma-GTase activity localized on the cell membranes and in the cytoplasm. From 44 wk carcinomas developed in 69% of the animals; they were predominantly well differentiated hepatocellular tumors and, to a lesser extent, hepatocholangiocellular carcinomas. Independent of histologic features, the tumors disclosed a markedly increased gamma-GTase activity both on tumor cell membranes and in the cytoplasm. During all preneoplastic and neoplastic stages, no gamma-GTase activity was seen in the parenchyma surrounding hyperplastic foci and carcinomas. A strong gamma-GTase activity in hyperplastic foci and subsequently in hepatocarcinomas further supported the commonly assumed precancerous nature of the former and illustrated the return of the latter to a more embryonic biochemical state. The fact that the focal reappearance of gamma-GTase occurred before the development of true carcinomas and then persisted in the tumor tissue indicates a close link between enzyme reappearance and neoplastic transformation, but does not clarify whether this was pathogenetic in the neoplastic process.

87. ENDOPLASMIC RETICULUM LAKES AND MALIGNANT HEPATOMA (LETTER TO EDITOR).

Theron JJ, Van der Watt JJ
Inst. for Cellular Physiology, Univ. Pretoria, Pretoria, South Africa
S Afr Med J; 50(12):461 1976

Intracytoplasmic globules formed by dilated cisternae of the rough endoplasmic reticulum (ER) were found in the normal and malignant hepatocytes of a human with hepatocelliver carcinoma and alpha1 -antitrypsin deficiency (AATD). These globules closely resembled those "ER lakes" found previously in mycotoxin-induced hepatoma cells in monkeys. This similarity suggests that in all future work on hepatocarcinogenic mycotoxins, the AAT levels be measured. The results of such studies may support the suggestion that AATD may promote malignant transformation of liver cells.

88. THE ULTRASTRUCTURAL EFFECTS OF AFLATOXIN B1 IN THE RAT PANCREAS.

Rao MS, Svoboda DJ, Reddy JK
Univ. Kansas Med. Cent., Kansas City, KS
Virchows Arch Zellpathol; 17(2):149-157 1974

The acute effects of aflatoxin B1 (AFB1) on the morphology of the acinar cells of the exocrine pancreas were studied in inbred male F-344 rats given one or two ip injections of 250 microg/kg AFB1. By light microscopy, the changes were unremarkable except for a slight decrease in the number of zymogen granules in animals given a single dose of AFB1 and a marked reduction of number in animals given two doses. By electron microscopy, the changes were: (a) a progressive decrease in the number of zymogen granules and by 48 hr irregularity of the limiting membrane, (b) dilation of Golgi vesicles at 24 hr, (c) dilation of endoplasmic reticulum and disaggregation of ribosomes at 24 hr, and (d) areas of focal cytoplasmic degradation at 48 and 72 hr. Marked mitochondrial changes in animals treated with two doses of AFB1 resembled those seen in livers of animals treated with AFB1 or carbon tetrachloride. The observed changes, though not specific, clearly suggest that AFB1 is a pancreaticotoxic agent.

89. CHEMICAL CARCINOGENESIS: EARLY BIOLOGICAL RESPONSES IN INDUCED CARCINOGENESIS OF THE KIDNEY. (PP. 34-38)

Butler WH, Hard GC
The Prediction of Chronic Toxicity from Short Term Studies, Proceedings of the European Society of Toxicology held at Montpellier, June 1975. Duncan WA, Leonard BJ, Brunaud M, ed. New York, American Elsevier Publishing Co Inc, Vol. 17, 1976.

Description is made of the morphological and histochemical features of premalignant kidney tissues after treatment of rats with an LD50 dose of dimethylnitrosamine (DMN) and of similarly premalignant livers during and after the feeding of rats with a diet containing 5 ppm of aflatoxin for 6 wk. In both instances, there is a continuous sequence of events leading from acute toxic injury to a chronic reaction that results in recognizable neoplasia. Following DMN treatment, an acute phase of about 2 wk is observed: after 24 hr, interstitial fibroblasts of the kidney cortex have autophagic vacuoles and other cytoplasmic abnormalities; a wave of proliferation of interstitial fibroblasts peaks after 6 days; and a peak inflammatory reaction occurs around the seventh day as a result of the necrosis of a few scattered proximal tubule cells. Persistent hypocellular foci, with abundant plasma cells, lymphocytes, and macrophages, are observed during a chronic reaction phase lasting from about 2 to 12 wk after treatment. After 12 wk, there is a rapid proliferation of mesenchymal foci that leads to unequivocal microscopic neoplasms after 20 wk. These observations suggest that the neoplasm is induced as an acute phenomenon, the cell of origin being an interstitial cortical fibroblast or, possibly, an extramural pericyte that retains its vasoformative properties. (13 Refs)

90. ADENOMATOUS POLYPS IN THE STOMACHS OF HATCHERY-GROWN SALMONIDS AND OTHER TYPES OF FISHES.

Kimura I, Miyake T, Kubota S, Kamata A, Morikawa S, Ito Y
Lab. Viral Oncology, Aichi Cancer Center Res. Inst., Nagoya, Japan
Prog Exp Tumor Res; 20:181-194 1976

The morphological characteristics and results of epizootiological and preliminary experimental studies of adenomatous polyps in the stomachs of hatchery-grown trout and in other species of fish are discussed. An outbreak of hepatoma was reported in hatchery-grown trout in Japan in 1969. The incidence was very high (100% at some stations) during 1970-1971, but it was down to 0-3% by 1973. Various observations and studies indicated that the main causative factor was aflatoxin B1 contamination of the fish meal. Adenomatous polyps were observed in the stomachs of hatchery-grown trout. The tumors developed multicentrically, mainly in the cardiac portion of the stomach, and they were composed of hypertrophic, mucus-secreting cells growing in papillary folds on stalks projecting into the gastric lumen. The incidence of the stomach polyps decreased during 1972 and 1973 relative to 1970-1971, the years of very high hepatoma incidences, suggesting that aflatoxin and/or its metabolites accelerated the development of these polyps. Some tumors, however, were seen when aflatoxin was absent from the diet, indicating that some other carcinogen may have been involved. One of the main factors in the development of stomach polyps seemed to be repeated mechanical injury, since their incidence was much lower in fish kept in trout ponds having beds of dead leaves compared to fish living in ponds containing large quantities of gravel. The incidence of stomach polyps was also very high in trout, yellowtails (Seriola quinqueradiata), and sea bream (Pagrus major) given commercial pellet food. (9 refs)

III. EFFECTS OF MYCOTOXINS ON MICROBIAL SYSTEMS AND CELL CULTURES

A. Mutagenic Action of Mycotoxins in Bacteria and Bacteriophages

91. AFLATOXIN MUTAGENESIS.

Ong TM
Natl. Inst. of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, N.C. 27709
Mutat Res; 32(1):35-53 1975

The chemistry, toxicity, carcinogenicity, teratogenicity, mutagenicity, and other genetically related activities of aflatoxins are reviewed. Twelve aflatoxin compounds are chemically recognized; these include aflatoxin B1 , aflatoxin B2 , aflatoxin G1 , aflatoxin G2 , aflatoxin B2a , aflatoxin G2a , aflatoxin M1 , aflatoxin M2 , aflatoxin R0 , aflatoxin Q1 , aflatoxin P1 , and aflatoxin GM1 . The toxic effect of aflatoxin has been demonstrated in many animal species; initial symptoms and relative toxicities are discussed. Aflatoxin carcinogenicity has been studied in various animals; both the lactone and the dihydrofurofuran portions of aflatoxin are necessary for the carcinogenic activities. Limited data on the teratogenic activity suggest a high degree of species specificity. Studies on the induction of chromosomal aberrations caused by aflatoxins have demonstrated abnormalities of chromosomal fragments, stickiness, bridges, chromatid breakage, and rearrangements. The most sensitive region is the heterochromatic area of the X chromosomes. The cytogenetic effects of aflatoxin in human WBC and in cells from rat kidney appear to be delay type, suggesting that aflatoxin is an alkylating agent. Studies on the induction of gene mutations have shown comparable relative frequencies of genotypes, allelic complementation, and nonpolarized complementation patterns induced by aflatoxin B1 and G1. Especially noteworthy is a quantitative relation of mutagenicity to interaction with DNA . While some studies on tumor induction have suggested that aflatoxin is active per se, others have indicated that metabolic activation is necessary. Tumor induction in only metabolically active cells, species-related teratogenicity, and definite organ specificity are cited. Other genetically related activities observed include induced phage production in lysogenic bacteria, inhibited DNA synthesis and inhibition of mitosis. The mutagenic hazard of aflatoxin to man is discussed in relation to aflatoxin distribution and contamination. A high cancer incidence has been found in certain geographic areas, and several studies have indicated that aflatoxin is responsible. Although direct evidence is still lacking, the induction of chromosomal aberrations in cultured human cells and the conversion of aflatoxin to mutagenic metabolites by human liver microsomes suggest a potential mutagenic hazard to man. (108 refs)

92. MOLDS, BACTERIA AND CANCER.

Garner C
Dept. Exp. Pathol., U. Leeds, England
New Sci; 63(909):325-327 1974

A bacterial mutagenicity assay method for studying the activity of aflatoxins has been developed. When aflatoxin B1, rat liver enzymes, and bacteria (strains of Salmonella species which required histidine for growth; mutated bacteria no longer need histidine) were incubated together at 37 C, the bacteria were rapidly killed. Only organisms deficient in the DNA repair enzyme were sensitive. This indicated that killing was due to attack on the bacterial DNA by a metabolite of aflatoxin. Aflatoxin B1 itself was not toxic to the bacteria, indicating that an aflatoxin B1 metabolite was the toxic agent. Of the other naturally occurring aflatoxins, only those that are potent carcinogens were metabolized by the liver enzymes to toxic and mutagenic derivatives. When liver from several other species was tested for ability to convert aflatoxin B1 to a toxic metabolite, it was found that all those tested, including human liver, could carry out this conversion. All aflatoxin-like molecules converted to toxic metabolites had a double bond at the 2,3-position. Such double bonds are converted to epoxides by liver enzymes. The epoxide formed during aflatoxin B1 metabolism is postulated to react with bacterial DNA to induce mutations and with mammalian cell macromolecules to initiate the cancer process.

93. MUTAGENICITY OF 3A,8A-DIHYDROFURO2,3-BBENZOFURAN, A MODEL OF AFLATOXIN B1, FOR SALMONELLA TYPHIMURIUM TA100.

Coles BF, Smith JR, Garner RC
Dept. Chemistry Univ. York, Heslington, Yorkshire Y01 5DD, England
Biochem Biophys Res Commun; 76(3):888-892 1977

The 3a,8a-dihydrofuro(2,3-b)benzofuran structure (I) occurs in a number of toxic, mutagenic, and carcinogenic natural products, notably the aflatoxins and sterigmatocystins. It is this function in aflatoxin B1 (AFB1) that is metabolically activated to yield the highly reactive epoxide AFB1-8,9-oxide, which may be responsible for the mutagenic and carcinogenic properties of AFB1. Compound I has been synthesized chemically and tested for its mutagenicity. In the presence of the 9,000 x g rat liver supernatant fraction, I induced his+ revertants in Salmonella typhimurium strain TA100 (but not strain TA98), but with only 1/5,000th of the activity of AFB1. Omission of the rat liver preparation abolished the mutagenicity entirely. It is suggested that one of the following factors may account for the great difference in mutagenicity between I and AFB1: reduced conversion to epoxide or inability of I to intercalate with DNA. (17 Refs)

94. THE SPORULATION SYSTEM OF BACILLUS SUBTILIS AS THE BASIS OF A MULTI-GENE MUTAGEN SCREENING TEST.

MacGregor JT, Sacks LE
Western Regional Res. Lab., Berkeley, CA 94710
Mutat Res; 38(4):271-286 1976

The sporulation system of Bacillus subtilis was used to detect the forward mutations in any of several hundred genes in 28-45 separate operons scattered throughout the chromosome. Nonsporulating or oligosporogenous mutant colonies were easily identified by their lack of a brown pigment normally present in spore-forming colonies. The dose dependence of sporulation mutant induction by 2-nitrosofluorene, ICR-191, nitrogen mustard, ethidium bromide, and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was demonstrated; mutagenesis was also demonstrated for aflatoxin B1 and 4-nitroquinoline-N-oxide. A mammalian liver enzyme metabolizing system was necessary to activate aflatoxin B1 . Auramine-O and 4-nitro-o-phenylenediamine failed to give a significant mutagenic effect under the conditions employed. The wide variety of mutagen classes detected indicates the general applicability of this unique and simple test. It is based on many genes throughout the chromosome, and therefore may prove less apt to exclude rare mutagenic hot-spots than systems based on the detection of mutations in a restricted region of the chromosome.

95. MUTAGENICITY OF AFLATOXINS RELATED TO THEIR METABOLISM AND CARCINOGENIC POTENTIAL.

Wong JJ, Hsieh DP
Department of Environmental Toxicology, University of California, Davis, Calif 95616
Proc Natl Acad Sci USA; 73(7):2241-2244 1976

Aflatoxins and their animal biotransformation products were screened for carcinogenic potential using the Ames' in vitro microbial detection system for carcinogens as bacterial mutagens (BN Ames et al (1973) Proc Natl Acad Sci USA 70, 2281-2285). Aflatoxicol , aflatoxins G1 and M1, aflatoxicol H1, and aflatoxins Q1, B2, P1, G2, B2a, and G2a, listed in order of decreasing mutagenic potency, were all less active than aflatoxin B1. No compound possesses activity in the absence of the rat liver preparation, and this indicates none of the animal metabolites are the ultimate mutagenic and/or carcinogenic species. The relative mutagenic potency observed with this in vitro system qualitatively correlates with in vivo carcinogenic data. Comparison of both methods indicates: (i) aflatoxin B1 possessed the structure optimal for both mutagenicity and carcinogenicity, (ii) the decreased carcinogenicity of various animal metabolites is associated with their decreased mutagenicity, and (iii) the 2,3-double bond is involved in both the mutagenic and carcinogenic activity of aflatoxins. The Ames' assay has been demonstrated to be an extremely promising (toxicological) tool for the analysis of mycotoxins for mutagenic and/or carcinogenic activity. (Author Abstract)

96. A COMBINED BACTERIAL AND LIVER TEST SYSTEM FOR DETECTION AND CLASSIFICATION OF CARCINOGENS AS MUTAGENS.

Ames BN
Biochem. Dep., Univ. California, Berkeley
Genetics; 78(1):91-95 1974

Carcinogens are detected and classified using Salmonella tester strains developed for mutagen detection and a rat (or human) microsomal hydroxylase system. Carcinogens detected as active frameshift mutants with this coupled system include aminobiphenyl, amino and dimethylamino transstilbene, 2-amino and 2-acetylaminofluorene, aminoanthrancene, benzidine, aminopyrene, aminochrysene, dimethylbenzanthracene, benzpyrene, and aflatoxin B1. The structural features of these carcinogens are discussed in relation to the theory of frameshift mutagenesis. Other carcinogens have been detected as mutagens causing base pair substitutions. It is concluded that carcinogens cause cancer by somatic mutation, and that compounds which give a positive response in the test should be considered potentially hazardous for man. A simple test for detecting mutagens in urine has also been developed.

97. ACTIVATION OF ENVIRONMENTAL CARCINOGENS BY HUMAN LIVER ENZYMES. (MEETING ABSTRACT).

Tang T, Friedman MA
Dept Microbiol, Va Commonwealth Univ, Richmond, VA 23298
Fed Proc; 36(3):304 1977

The in vitro short-term Ames mutagenicity test employs rodent liver enzymes as a metabolic source of carcinogen activation. This test shows a 90% correlation between mutagenicity and carcinogenicity in rats of a myriad of compounds. Using the Ames test, we have evaluated the ability of 7 human liver samples to activate 10 environmental carcinogens including 4 aromatic amines, a nitro-aromatic compound, 2 polycyclic hydrocarbons, 2 mycotoxins and an azo dye. Salmonella typhimurium TA100 was used in these studies. Two liver samples were obtained from brain-dead donors and 5 samples were from autopsy cases. The age of the individuals ranged from 3-days-old to 92-years-old. The ability of these samples to activate aromatic amines ranged from not active to highly active as exhibited by one sample for 2-acetylaminofluorene. Five samples were highly active for p-nitrobiphenyl. All samples showed a generally low activity in activating 3-methylcholanthrene and benzo(a)pyrene. **The ability to activate aflatoxin B1 and sterigmatocystin was the highest among all compounds tested** and approximately equal. These results indicated that there is a substantial variation in the ability of carcinogen activation by human liver enzymes derived from different individuals. (Author Abstract)

98. DIURNAL OSCILLATION OF RAT LIVER MEDIATED MUTAGENESIS BY AFLATOXIN B1 (MEETING ABSTRACT).

Pariza MW, Rappaport H, Garcia VM, Goepfert JM
Food Res Inst, Dept of Food Microbiology and Toxicology, Univ of Wi, Madison, Wi. 53706
Proc Am Assoc Cancer Res; 18:26 1977

We have employed the Ames salmonella mutagenesis assay to study the regulation of carcinogen metabolism by rat liver. Male Sprague-Dawley rats were adapted to a controlled lighting-and-feeding schedule consisting of 12 hr of light followed by 12 hr of darkness with food available only during the first 2 hr of the dark period. Animals (3/group) were killed at 6 hr intervals. Livers were rapidly excised, homogenized, and 9000xg supernatants (S-9 fractions) prepared and assayed for the capacity to produce revertant colonies of S. typhimurium TA1538 following exposure to S-9 fractions plus aflatoxin B1 (B1) or 2-aminofluorene (AF). In experiments involving B1, S-9 fractions obtained 6 hr into the light period were twice as active as S-9 fractions obtained at the beginning of the light period. S-9 fractions obtained during the dark period displayed intermediate levels of activity. In contrast, parallel experiments with AF produced similar numbers of revertant colonies regardless of the time of day that S-9 fractions were obtained. (Author Abstract)

99. DETECTION OF CARCINOGENS AS MUTAGENS: BACTERIAL TESTER STRAINS WITH R FACTOR PLASMIDS (MEETING ABSTRACT).

McCann J, Spingarn NE, Kobori J, Ames BN
Biochem, Dep., Univ. California, Berkeley
Proc Natl Acad Sci USA; 72(3):979-983 1975

The detection of carcinogenic agents as mutagens in bacterial test strains with an R factor plasmid was investigated. R factor transfer was carried out using R-factor-containing donor strains and recipient strains diluted in nutrient broth. Ampicillin -resistant recipients were selected on petri plates containing ampicillin and required supplements (histidine and biotin). Donor strains SL1156 (trpD1/R Utrecht), SL1127 (pur pro/R46) and TA2000 (purF145/KM101) were produced by transforming pKM101 from hisG46/pKM101 to purF145. R factor and tester strains were maintained at -80 C after freezing nutrient broth with dimethyl-sulfoxide . The R factor plasmid in bacterial strains developed two new strains (TA100 and TA98) which significantly increased the number of carcinogens as mutagens. Aflatoxin B , sterigmatocystin benzoa-pyrene, 7,12-dimethylbenzanthracene were carcinogenic mutants more readily detected with the two new strains. HisG46 containing pKM101 and TA100 (TA135 with pKM101) was most sensitive to methyl methanesulfonate -induced reversion and pSC101 was found to be inactive. Mutagens found in this study seemingly worked more effectively due to an error-prone recombinational repair of DNA . Further investigation into the mechanism of the R plasmid enhancement of mutagenesis is indicated.

100. IN VITRO SYSTEMS USING MICRO-ORGANISMS FOR DETECTION OF CARCINOGENIC AGENTS.

Garner RC
Chemotherapy. Hellmann K, Connors TA, ed. New York, Plenum Press, vol. 7, 1976.

An outline is given of a proposed system to screen for chemical mutagens/carcinogens that utilizes lambda bacteriophage as a detector organism. Preliminary work has demonstrated a dose-dependent relationship for survival of the phage after in vitro treatment with 2-acetoxy-2-acetylaminofluorene (AAAF) and after in vitro treatment with aflatoxin B1 in the presence of a microsomal activating system. Greater inactivation of the phage occurred during assay with the uvrA and lambda red strains of Escherichia coli than with wild-type E coli. A similar dose-dependent relationship was demonstrated when clear plaque mutation, a forward mutation (unlike the back mutations that provide the basis of the Ames' Salmonella screening system), was assayed for after treatment with AAAF or 7-bromomethylbenz(a)anthracene. The phage assay has the potential for responding to the organ-specific activation of carcinogens in vivo: phage can be recovered from liver, lungs, spleen, kidney, and blood 24 hr after ip injection; phage phagocytosed by the reticuloendothelial system persist as viable particles for at least 1 wk. (16 refs)

101. PROPHAGE LAMBDA INDUCTION IN ESCHERICHIA COLI K12 ENVA UVRB: A HIGHLY SENSITIVE TEST FOR POTENTIAL CARCINOGENS.

Moreau P, Bâilone A, Devoret R
Section de Radiobiologie Cellulaire, Laboratoire d'Enzymologie, C.N.R.S., 91190 Gif-Sur-Yvette, France
Proc Natl Acad Sci USA; 73(10):3700-3704 1976

A simple, inexpensive, and sensitive test for potential carcinogens based upon the property of carcinogens to induce prophage lambda is described. By using chemicals activated with microsomal enzymes and E coli K12 permeable (envA) tester bacteria also deficient in DNA repair (uvrB), the range of carcinogens detected in a lysogenic induction test (inductest) has been extended. We have provided the evidence that, after activation, carcinogenic polycyclic hydrocarbons such as benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene induce prophage lambda. Three variants of the test have been developed (inductests I, II, and III), which are as sensitive as the mutagenicity test of Ames et al. (Ames, B N, McCann, J and Yamasaki, E (1975) Mutat Res; 31, 347-364). Inductests II and III provide a quantitative estimation of the inducing activity of a carcinogen. With the latter test, one can determine: (i) the cellula toxic effect of a carcinogen and (ii) the kinetics of appearance and disappearance of active metabolites. For two series of chemicals, aflatoxins and benz(a)anthracenes, there is a good correlation between their carcinogenic activity in rodents and their prophage inducing activity in bacteria. The fact that the majority of the cell population is induced makes it possible to test the inducing activity of carcinogens at the biochemical level, e g, by measuring lambda repressor inactivation. (Author Abstract)

102. INDUCED REACTIVATION OF UV-DAMAGED PHAGE LAMBDA IN E. COLI K12 HOST CELLS TREATED WITH AFLATOXIN B1 METABOLITES.

Sarasin A, Goze A, Devoret R, Moule Y
Dept. Biological Sciences, Stanford Univ., Stanford, CA 94305
Mutat Res; 42(2):205-213 1977

Metabolites of aflatoxin B1 (MAB) were prepared by incubation with the 9,000 g supernatant of the livers of rats previously treated with sodium phenobarbital plus an NADPH-generating medium. MAB promoted the reactivation of phage lambda damaged by radiation, in Escherichia coli K12 cell lines. The reactivation process was found to be error prone: 25% of the phage DNA lesions were repaired, but mutagenesis, scored as clear plaque formation, increased as much as tenfold. Such reactivation of UV-damaged phage lambda, which occurred in wild-type and in uvrA but not in recA bacteria, was inducible: phage reactivation was obtained even after a long delay following treatment of the host by MAB. This induced reactivation of UV-damaged phage in hosts treated with MAB is similar to direct or indirect UV reactivation. MAB-induced phage produced induced phage reactivation as well as prophage lambda induction in lysogens and cell filamentation in nonlysogens. These cellular events were also triggered by DNA lesions caused by UV radiation and result from the induction of a metabolic pathway (the previously described SOS functions). It is postulated that, in eukaryotes, carcinogens may induce SOS functions similar to those in E. coli. (29 Refs)

103. MUTATION INDUCTION BY RODENT LIVER MICROSOMAL METABOLITES OF AFLATOXINS B1 AND G1 IN NEUROSPORA CRASSA.

Matzinger PK, Ong TM
Environmental Mutagenesis Branch, Natl. Inst. Environmental Health Sciences, Research Triangle Park, NC 27709
Mutat Res; 37(1):27-32 1976

The mutagenic activities of aflatoxin B1 and G1 were studied in the ad-3 test system of Neurospora crassa by treatment of conidia with aflatoxin and hamster liver homogenates for 2 hr. There was no significant increase in ad-3 mutation frequency when either aflatoxin or mammalian liver homogenate was omitted. The mutation frequencies increase to between 29/10^6 and 87/10^6 survivors; this is a 73-to 217-fold increase over the av spontaneous ad-3 mutation frequency (0.4/10^6 survivors) after conidia of N crassa were treated with 0.67 milliM aflatoxin B1, liver homogenate, and a NADPH generating system. There was a 9-to 15-fold increase in mutation frequency when 0.67 milliM of aflatoxin G1 was used. Treatment with 0.44 milliM aflatoxin B1 mouse liver homogenate and a NADPH generating system induced a small, but significant, increase in mutations. A single sample of human liver homogenate caused no mutations. These data indicate that metabolic activation is necessary for the mutagenic activity of aflatoxins B1 and G1 in N crassa. (16 Refs)

104. IN VITRO ACTIVATION OF AFLATOXIN B2 TO METABOLITES MUTAGENIC IN NEUROSPORA CRASSA (MEETING ABSTRACT).

Matzinger PK, Ong TM
Natl. Inst. Environmental Health Sciences, Res. Triangle Park, NC
Mutat Res; 38(2):121 1976

Aflatoxin B1 , a potent hepatocarcinogen, was not mutagenic in Neurospora crassa when the metabolically inactive resting conidia were treated. Therefore, studies were conducted to determine whether aflatoxin B1 could be activated by mammalian liver microsomes to metabolites mutagenic in N crassa. The ad-3 test system of N crassa was used - with this system, recessive lethal mutations resulting from point mutations or chromosomal deletions can be recovered at loci ad-3A and/or ad-3B. Conidia from a genetically marked two-component heterokaryon were treated with aflatoxin B1 , mouse or hamster liver microsomes and a NADPH-generating system. After a 2-hr treatment at 37 C, the chemicals were decanted by centrifugation; the conidia were washed and resuspended in Frie's basal medium, pH 8. Treated and untreated conidia were analyzed for the presence of a ad-3 mutants by the direct method. The results indicated that when resting conidia were treated with 0.67 mM of aflatoxin B1 in the presence of hamster liver microsomes and a NADPH-generating system, the ad-3 mutation frequency was increased to between 29.3 and 87.1 per 10^6 survivors, which was a 76- to 230-fold increase over the spontaneous frequency (0.38 x 10**-6). Only a seven- to ninefold increase over the spontaneous frequency was observed when mouse liver microsomes were used. No increase in mutation frequency could be detected without microsomes. Apparently metabolic activation is necessary for expression of the mutagenic activity of aflatoxin B1 in N crassa. (No refs)

B. Responses of Cell Cultures to Mycotoxin Treatment

105. AFLATOXIN-INDUCED CHANGES IN HUMAN HEPATOCYTES IN ORGAN CULTURE.

Noyes WF
Roswell Park Memorial Institute, Buffalo, N. Y. 14203
Proc Am Assoc Cancer Res; 16:26 1975

The association between aflatoxin contamination of food and the incidence of primary carcinoma of the liver in areas of Africa and Thailand (Alpert et al, Cancer 28, 253, 1971) (Shank et al, Fd Cosmet Toxicol, 10, 171, 1972) stimulated investigation of the effect of purified aflatoxin on adult human liver parenchymal cells maintained in long-term organ culture. Adult human liver obtained at operation was cut into 1-2 mm fragments and cultivated on sponge in McCoy's supplemented with serum. Aflatoxin B1 was dissolved in acetone containing 10% water and further diluted in McCoy's. Organ cultures were fixed in Bouin's and serially sectioned. Explants of adult human liver were cultured for up to 5 weeks with maintenance of the hepatic parenchymal cells. Exposure of the organ cultures to aflatoxin B1 at a concentration of.01 microgram/ml resulted in changes in morphology of some of the parenchymal cells and at a concentration of 1 microgram/ml areas of parenchymal cells with markedly altered morphology were observed. The cells in these areas were enlarged with pale-eosinophilic cytoplasm and hyperchromatic nuclei, similar to the altered hepatic parenchymal cells of rats fed a diet containing aflatoxin B1 described by Newberne and Wogan (Cancer Res., 28, 770, 1968). (Author Abstract)

106. ESTABLISHMENT OF A HUMAN HEPATOMA CELL LINE (MEETING ABSTRACT).

Alexander J. Bey E

Cancer Res. Unit Natl. Cancer Assoc., Poliomyelitis Res. Foundation, Johannesburg, South Africa

S Afr Med J; 50(26):1019-1020 1976

The high incidence rate of a particular cancer in any region is indicative of a viral and/or chemical aetiology. Epidemiological studies have suggested aflatoxin as a causative agent in primary liver cancer, and associations between hepatoma and hepatitis B antigen (HB8 Ag) have been documented. Alpha-fetoprotein (AFP) at high levels has been measured in the sera of hepatoma patients. The aim of this study was to produce a cell line from human hepatoma material in order to (1) study the biochemistry of the cells in pure culture, (2) detect the presence of any indigenous virus or antigens, and (3) quantitate the cytotoxic effect of various chemicals on these cells. Seven separate hepatoma specimens were prepared for tissue culture and one has been established as a permanent cell line (PLC/PRF/73). The tumour was taken in 1973 from a patient whose serum was positive for both HB8 Ag and AFP. The hepatoma cells in tissue culture grew slowly for 9 mo and adaptation of in vitro conditions were completed after 18 mo. The morphology of the cells has not altered since initial isolation, and electron microscopy of both the original tumour and the cells in vitro has not shown the presence of defined particles. Recently sufficient material was produced for a variety of biochemical and immunological tests. The cells are more resistant to aflatoxin B1 than are normal cells, but other tumour cells in tissue culture show approximately equal sensitivity. Dr. Macnab (SAIMR) has shown from her investigations that AFP is not produced in supernatant culture fluids from this cell line but that the latter is capable of HB8 Ag excretion. The results of her studies will be presented in the ensuing paper.

107. CHROMOSOME BANDS INDUCED IN HUMAN AND SYRIAN HAMSTER CELLS BY CHEMICAL CARCINOGENS.

DiPaolo JA, Popescu NC

Natl. Cancer Inst., Bethesda, Md.

Br J Cancer; 30(2):103-108 1974

After 24 hr of treatment of human peripheral leucocyte cultures or Syrian hamster embryonic secondary cultures with some known chemical carcinogens (7,12-dimethylbenz(a)anthracene, benzo(a)pyrene, aflatoxin B1, N-acetoxy-2-fluorenylacetamide, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitroquinoline-1-oxide and ethylcarbamate) at concentrations that produce transformation in vitro of hamster cells, the chromosomes of both types of cells exhibited typical G banding. Chromosome bands did not result with urethane, a compound which does not produce cytotoxicity or cause transformation by direct exposure of cells. Noncarcinogenic chemicals which do not inhibit cell multiplication also failed to produce bands. Cytogenetic analysis following removal of the carcinogens from the cultures indicated non-banded uniformly stained chromosomes. It is concluded that metaphases with chromosome bands are more likely to be the result of nonspecific toxicity rather than being related to the carcinogenic properties of the chemicals.

108. CYTOTOXIC ACTIVITY OF MACROCYCLIC METABOLITES FROM FUNGI.

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Neoplasma; 24(1):21-27 1977

The effect of the following fungal metabolites on HeLa cells was studied: cyanein, cytochalasins A, B, and D, zygosporin A, curvularin, monorden, and zearalenone. Each substance was tested in six concentrations and their effect was characterized by the values of ED50 and/or ED100 (concentration required for 50% and/or 100% suppression of cell growth, respectively). There were great differences in cytotoxicity. Curvularin and zearalenone were least effective, with ED50 at 2 x 10**-4 and 7.5 x 10**-5 M, respectively. Most effective were cyanein and monorden with ED50 at values at a thousandfold lower concentration than that of zearalenone. The ranges of ED50 and ED100 for cytochalasin A, B, and D were between 1.2 and 2.1 x 10**-6 M and between 2.4 and 5.2 x 10**-6 M, respectively. Thus, there were three categories of metabolites: those with very high cytotoxicity (cyanein and monorden), those with marked cytotoxicity (cytochalasins), and those with very little activity (zearalenone and curvularin). These cytochalasins are structurally similar to each other; their differences, in the macrocyclic parts of the structures, do not significantly influence their cytotoxicity but do result in different activity on microorganisms. Differences in cytotoxicity of monorden, zearalenone, and curvularin are related to differences in their chemical structures, specifically in the structure of the macrocyclic ring. The highest degree of conjugation is seen in monorden, the lowest in curvularin. However, the significance of the number or the position of hydroxyl groups at the benzene nucleus, or of the presence of a chlorine atom in the monorden molecule, is still unclear. (13 refs)

109. EFFECT OF AFLATOXINS ON ANIMAL CELLS IN TISSUE CULTURE.

Beniumovich MS

Inst. Nutr., Moscow, USSR

Farmakol Toksikol; 36(4):497-501 1973

Literature on the following aspects of the use of tissue culture for the study of properties of aflatoxins is reviewed: (1) production of aflatoxin preparations and method of introducing them into cultures; (2) characteristics of cultures used to study the effects of aflatoxins; (3) toxicity of aflatoxins in tissue culture; (4) characteristics of changes caused by aflatoxins in cultured cells and mechanisms of aflatoxin action; (5) specificity of the toxic effect of aflatoxins in cell cultures, and (6) comparison of data obtained in vivo and in tissue culture. Experiments with cell and tissue culture indicate high sensitivity to the effect of aflatoxins in populations of rapidly propagating cells, although the sensitivity of cells of various stable lines varies to a known degree. Aflatoxin-induced damage occurs primarily in cells undergoing mitosis. Hepatocytes are apparently more sensitive to aflatoxins in vitro than cultured cells of other organs. However, under conditions of retarded propagation (primary trypsinized cultures, diploid lines) the resistance of hepatocytes and their derivatives to aflatoxins is probably quite high. The in vitro effect of aflatoxins on nonpropagating liver cells requires further study. Research on the use of tissue culture methods for study of the selective sensitivity of liver cells to aflatoxins is of considerable interest. Aflatoxins are not known to have a carcinogenic effect on cultured cells.

110. CHEMICAL CARCINOGEN AND LIPOPEROXIDATIVE EFFECTS ON THE RIBOSOME MEMBRANE COMPLEX OF ROUGH MICROSOMAL MEMBRANES (MEETING ABSTRACT).

Shires TK

The Toxicology Center, Depts of Pathology and Pharmacology, The Univ of Iowa, Iowa City, IA 52242

Fed Proc; 36(3):1078 1977

Chemical carcinogens like dimethylnitrosamine (DMNA) and aflatoxin b (AFTX) cause an in vivo decline in the amount of rough endoplasmic reticulum in hepatocytes. From various studies both in vivo and in vitro, it has been deduced that carcinogens promote detachment of ribosomes from the rough membranes (Rmic). We have isolated Rmic from rat liver, incubated it with either DMNA or AFTX and by density gradient centrifugation assayed for ribosomal detachment. Using incubation systems that demonstrably supported metabolism of carcinogen or amino acid incorporation in the presence of carcinogen, no evidence for ribosomal detachment was found, nor was there detachment after concurrent metabolism and incorporation. However, if lipoperoxidation was allowed to occur in the metabolizing system by adding increased amounts of NADPH (1 mM) giving 15-20 nmoles malondialdehyde /mg, ribosomes appeared to detach but, in so doing, carried much Rmic phospholipid with them. If lipoperoxidation was restrained with antioxidant no detachment occurred unless K**+ concentration was elevated to 750 mM. The presence of carcinogen was not required for lipoperoxidation, the NADPH was alone a sufficient pro-oxidant. While even minimal lipoperoxidation alters the domain of the binding site, detachment does not seem a likely explanation for carcinogen-promoted decline in the extent of liver rough membranes in vivo. (Author Abstract)

111. CERTAIN ASPECTS OF CHEMICAL CARCINOGENESIS IN VITRO USING ADULT RAT LIVER CELLS. (PP. 425-440)

Iype PT, Allen TD, Pillinger DJ
Gene Expression and Carcinogenesis in Cultured Liver.
Gerschenson LE, Thompson EB, ed. New York,
Academic Press Inc, 1975.

Chemical carcinogenesis was studied in vitro using well-characterized adult rat liver cells. The cells were isolated from 6- to 8 wk-old Wistar rats using the collagenase and hyaluronidase perfusion method and plated in Ham's F10 medium supplemented with 10% fetal bovine serum. The plated cells were polygonal in shape and highly granular; many were binucleate. Cells from areas in the dishes that were rich in granular cells were preferentially isolated, pooled, and cultured. Binucleate cells were not seen after a few days in culture. Light and electron microscopy indicated that these cells were epithelial in their morphology and clearly distinct from hepatoma cells in culture; they exhibited contact inhibition of growth. These characteristics were maintained during several months in continuous culture. Confluent monolayers of the cell lines were treated with dexamethasone phosphate or hydrocortisone 21-sodium succinate at a concentration of 10**-5 M for 24 hr, and tyrosine aminotransferase (TAT) activity was assayed. The TAT activity of the established cell lines was 2.3-4.3 U in the various media used, values comparable to those of a hepatoma cell line. When the hepatoma cell line was treated with hydrocortisone 21-sodium succinate, TAT activity increased seven times, but it was not possible to induce TAT in the normal established cell lines. These rat liver parenchymal cell lines did not show any of the known properties of transformed cells and did not produce tumors when injected into syngeneic rats. Forty to 50 days following treatment with a nontoxic dose (50-100 ug/ml) of N-methyl-N-nitrosourea for 2 hr, some of the dishes showed foci of highly refractile cells that had the capacity to grow in soft agar. **Aflatoxin (0.5 ug/ml) produced toxic effects in the cells.** Three weeks after this treatment, multinucleated cells were seen that formed soft agar colonies. MNU-treated cell lines elicited antibody production (membrane immunoflourescence tests) when injected into a highly inbred strain of rats and induced undifferentiated carcinomas in the lung 1-2 mo after iv injection into rats. It is concluded that these studies demonstrate chemical carcinogenesis in vitro. (27 refs)

112. THE STUDY OF CHEMICAL CARCINOGENESIS USING CULTURED RAT LIVER CELLS. (PP. 480-487)

Williams GM
Gene Expression and Carcinogenesis in Cultured Liver.
Gerschenson LE, Thompson EB, ed. New York,
Academic Press Inc, 1975.

Chemical carcinogenesis in cultured rat liver cells was studied by determining single-strand breaks in DNA induced by carcinogens. The studies used epithelial cultures started from adult rat liver dissociated after perfusion with collagenase and hyaluronidase. The cellular DNA was labeled by adding 0.15 uCi/ml ^{3} H-thymidine to sparse cultures 24 hr after seeding. The cultures were grown to confluency in the presence of ^{3} H-thymidine and then switched to a control medium. The cells were subjected to alkaline sucrose gradient centrifugation sufficient to sediment the bulk DNA of control cells, and the smaller molecular weight broken DNA of the treated cells was distributed in the gradient. Nitrosomethylurea produced a dose-related fragmentation of DNA that was maximal at 15 min after treatment. Further incubation after maximal fragmentation revealed a shift of the sedimentation profile toward that of the bulk DNA, indicating fragments of higher molecular weight due to repair of some of the DNA damage. After 24 hr incubation, there was almost complete restoration of the normal sedimentation profile. In other studies, direct-acting carcinogens, such as N-acetoxyfluorenylacetamide and methyl methanesulfonate, readily produced breaks, but procarcinogens requiring metabolism gave equivocal or negative results. In view of the observed rapid repair, it was hypothesized that a low level of activation might result in so little damage to DNA that the cell could repair it as fast as it occurred, resulting in the appearance of no damage. Chloroquine (an inhibitor of DNA repair; 0.4 mM) was added to the cultures for 1 hr prior to treatment with carcinogens and allowed to remain for 4 hr of carcinogen treatment. Treatment with 2 x 10**-5 M dimethylnitrosamine, N-2-fluorenylacetamide, aflatoxin B1 , and dimethylbenzanthracene by themselves yielded no breaks. In the presence of chloroquine, however, breakage was obtained with all four procarcinogens. These preliminary data suggest that the cultures can generate a genotoxic product from the procarcinogens and that treatment with chloroquine may make possible the detection of low levels of activation. If this proves to be the case, this system would provide a simple, inexpensive, rapid assay for screening potential carcinogens. (13 refs)

113. A DIPLOID RAT LIVER CELL CULTURE. I. CHARACTERIZATION AND SENSITIVITY TO AFLATOXIN B1.

Buasher J, Schaeffer WI
Coll. Med., Univ. Vermont, Burlington
In Vitro; 9(4):286-293 1974

A method was described for culturing and cloning a diploid epitheloid cell line (tyrosine aminotransferase-synthesizing) from rat liver and the response of this culture and established cell lines (Chang liver and R-L-T) to aflatoxin B1 was studied. Rat liver cell lines (RL-NB, RL-PR-C) were derived from three-day-old rats. The diploid culture was more resistant to aflatoxin B1 (up to 50 microg) than the established cell lines. This was determined by cell counts, residual protein, and the incorporation of 14Curidine. It was suggested that the resistance of the diploid cell line was the result of in vitro acquired properties, or the result of an intrinsic property of a segment of the total cells present in the intact organ.

114. MAINTENANCE OF CYTOCHROME P-450 AND METABOLISM OF AFLATOXIN B1 IN PRIMARY HEPATOCYTE CULTURES.

Decad GM, Hsieh DP, Byard JL
Dept. Environmental Toxicology, Univ. California, Davis, CA 95616
Biochem Biophys Res Commun; 78(1):279-287 1977

Primary hepatocyte cultures isolated from male Sprague-Dawley rats were maintained in a defined medium containing several hormones that would enable the cells to maintain an in vivo level of cytochrome P-450. Aflatoxin B1 (AFB1) was then added to the cultures and its metabolism was studied to determine whether the data obtained could be extrapolated to the in vivo state. These hepatocyte cultures completely metabolized AFB1 in 10 hr, producing mostly water-soluble metabolites. In agreement with in vivo results, AFB1 was the only nonpolar metabolite found. A significant portion of AFB1 was covalently bound to cell constituents, which may be the basis for its toxicity and carcinogenicity. It is concluded that primary hepatocytes, in a defined culture medium supplemented with hormones, are a good model of the liver in studying the metabolism and toxicology of environmental carcinogens such as AFB1. (18 Refs)

115. TRANSFORMATION OF THE FISCHER RAT EMBRYO CELL SYSTEM BY TWO CARCINOGENIC MUTAGENS (MEETING ABSTRACT).

Auletta AE, Suk WA
Microbiological Assoc, Bethesda, MD 20016,
Proc Am Assoc Cancer Res; 18:137 1977

N-methyl-N-nitrosourea (MNU) and aflatoxin B1 are known mutagens/carcinogens which have not previously been shown to transform cells in culture. We report here the ability of both of these agents to transform the Fischer rat embryo cell system (F1706). A reduction in plating efficiency (PE) relative to a medium control was used to determine the toxicity of each agent. Treatment with 1 mug of aflatoxin B1 per ml of medium resulted in a 50% reduction in PE of F1706 cells. Morphological transformation was observed with 0.01 and 0.05 mug of aflatoxin B1 per ml of medium. Treatment of F1706 cells with 0.001 mug/ml of MNU resulted in a 38% reduction in PE; morphological transformation was observed with 0.005 and 0.01 mug of MNU per ml of medium. Transformation was determined by the appearance of spindle-shaped cells lacking contact inhibition and by the growth of transformed cells in semi-solid agar. The detection of these two compounds as transforming agents underscores the use of the F1706 system as a screen for potential carcinogens and highlights the correlation between mutagenicity, transformation of cells in culture and carcinogenicity in vivo. (Author Abstract)

116. NEOPLASTIC TRANSFORMATION OF GUINEA PIG FETAL CELLS IN CULTURE INDUCED BY CHEMICAL CARCINOGENS.

Evans CH, DiPaolo JA
Natl. Cancer Inst., Bethesda, Md.
Cancer Res; 35(4):1035-1044 1975

Relationships between various cell properties and tumorogenic potential were studied in 24 cell strains derived from freshly isolated diploid strain 2 Sewall-Wright guinea pig fetal cells exposed in utero or directly in culture to a carcinogenic or a noncarcinogenic chemical during 4-24 months of continuous culture. Carcinogenic agents included benzo(a)pyrene , 7,12-dimethylbenz(a)anthracene , 3-methylcholanthrene , N-methyl- N'-nitro-N-nitrosoguanidine , diethylnitrosamine , aflatoxin B1 , and N-acetoxyacetylaminofluorene . Noncarcinogenic chemicals were acetone , pyrene , anthracene , and phenanthrene . Morphological alternations (including various degrees of loss of cell orientation) were seen in the cultures exposed for five days to carcinogens but not in controls; however these morphologic changes did not appear until four or more months, and did not appear simultaneously with the capacity of transformed cells to grow as tumors in irradiated syngeneic newborn animals. Changes in plating efficiency, saturation density, doubling time, or chromosome alterations did not correlate with progressive tumor growth. Formation of colonies in 0.35% agar, however, did develop concurrently with the potential for neoplastic growth, and of all the cell properties examined it was the best indicator of the neoplastic state. Four to 18 months of culturing was required after exposure to the carcinogen.

117. PROTECTIVE EFFECT OF DEHYDROEPIANDROSTERONE AGAINST AFLATOXIN B1- AND 7,12-DIMETHYLBENZ(A)ANTHRACENE-INDUCED CYTOTOXICITY AND TRANSFORMATION IN CULTURED CELLS.

Schwartz AG, Perantoni A
Fels Research Institute and Department of Microbiology, Temple University Medical School, Philadelphia, Pennsylvania 19140
Cancer Res; 35(9):2482-2487 1975

Dehydroepiandrosterone, an adrenal steroid that has been reported to be produced in subnormal amounts and found in lower plasma concentrations in women with benign and malignant breast tumors, protects cultured rat liver epithelial-like cells against aflatoxin B1 induced cytotoxicity and protects hamster embryonic fibroblasts against aflatoxin B1 and 7,12-dimethylbenz(a)anthracene induced transformation. Related androgenic steroids show significantly less protective effect. Dehydroepiandrosterone also inhibits the rate of conversion of (^{3}H)-7,12-dimethylbenz(a) anthracene to water-soluble metabolites by rat liver epithelial-like cells, suggesting that the steroid probably exerts its protective effect by inhibiting carcinogen activation. (Author Abstract)

118. LIVER-HOMOGENATE MEDIATED MUTAGENESIS IN CHINESE HAMSTER V79 CELLS BY CHEMICAL CARCINOGENS.

Krahn DF, Heidelberger C
McArdle Lab., Univ. Wisconsin, Madison, WI 53706
Mutat Res; 38(6):399-400 1976

Chinese hamster V79 cells were treated with a number of diverse carcinogens in the presence of the 9000 gravity units supernatant fraction of rat liver and the cofactors, NADPH and NADH. Determination was made of the concentration of carcinogen (T nonometer ml) that reduced the plating efficiency of the cells by 50%, and the fold induction (M) of thioguanine-resistant variants over control levels. 'T' and 'M' were respectively: 0.3 and 10 for aflatoxin B1, and 35 and 2 for aflatoxin B2, with supernatant from untreated rats; 16 and 10 for benzo(a)pyrene, and 30 and 4 for benz(a)anthracene, with supernatant from 3-methylcholanthrene-treated rats; 13,000 and 200 for dimethylnitrosamine with supernatant from phenobarbital-pretreated rats. Cytotoxicity, and an increased frequency of thioguanine-resistant variants, has also been observed with 7,12-dimethylbenz(a)anthracene, methylcholanthrene and 2-naphthylamine. It is thus demonstrated that chemical carcinogens that require metabolic activation can be screened for mutagenic activity in cultured mammalian cells that are deficient in mixed function oxidase activity. (no refs)

119. LIVER HOMOGENATE-MEDIATED MUTAGENESIS IN CHINESE HAMSTER V79 CELLS BY POLYCYCLIC AROMATIC HYDROCARBONS AND AFLATOXINS.

Krahn DF, Heidelberger C
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI 53706
Mutat Res; 46(1):27-44 1977

A mammalian cell culture mutagenesis assay using Chinese hamster V79 cells, which are sensitive to the cytotoxic and mutagenic effects of several chemical carcinogens that require metabolic activation, is described. The induced frequency of 6-thioguanine-resistant colonies was used to measure mutagenic activity. The 9000-g supernatant fraction of rat liver plus cofactors provided the metabolic activation. Eventually, the assay could be utilized to prescreen environmental chemicals. The following chemical carcinogens were examined: aflatoxin B1, aflatoxin B2, benzo(a)pyrene, 3-methylcholanthrene, 7,12-dimethylbenz(a)anthracene, dibenz(a,h)-anthracene, dibenz(a,c)anthracene, and benz(a)anthracene. Except for dibenz(a,h)-anthracene and dibenz(a,c)anthracene, the mutagenic activity generally paralleled the carcinogenic activity. (64 Refs)

120. CHEMICAL CARCINOGENS STIMULATE CANINE KIDNEY (MDCK) CELLS TO PRODUCE PROSTAGLANDINS.

Levine L
Dept. Biochemistry, Brandeis Univ., Waltham, MA 02154
Nature (Lond); 268(5619):447-448 1977

Treatment of MDCK canine kidney cells with several carcinogenic aromatic hydrocarbons, aflatoxin B1 and 1,2,7,8-diepoxyoctane increased prostaglandin production relative to carcinogenicity of the chemical. The MDCK line could prove to be a sensitive indicator for the carcinogenic potential of hydrocarbons. (13 Refs)

121. THE DYNAMICS OF INTRACELLULAR METABOLIC TOPOGRAPHY (MEETING ABSTRACT).

Kohen E, Kohen C, Thorell B
Papanicolaou Cancer Res Inst, Miami, Fla 33136
Fed Proc; 36(3):798 1977

Rapid multisite topographic (and spectral) analysis of NAD(P) to and from NAD(P)H transients in cells (EL2,L) microinjected with metabolites (eg glucose-6-P) allows the definition of rate equations throughout the compartments of a living cell (up to approx 100 sites per cell). From the sequentially induced concentration-dependent transients a rate vs concentration relationship can be derived for each cell site, based on the halftimes of rise and decay(t1/2 off). Practically for all sites (cytoplasmic vs nuclear) exponential or power relationships are found. By varying the microinjected metabolite, allosteric factor (eg adenine nucleotides) or addition of metabolizable compounds (eg hydrocarbons, aflatoxins), rate equations can be defined for NAD- or NADP-linked pathways of degradation or synthesis throughout the living cell. A method is acquired for unraveling the microtopographic homogeneity or discrepancies of enzyme reactions throughout the living cell and its compartments. The Pasteur effect prevents the formation of glucose-6-P transients in L cells exposed to oxygen. The interplay of mitochondrial-extramitochondrial compartments is unraveled by growth in presence of oligomycin (1muM,24-48h) or atractylate (3muM,10-30 days), which suppresses the Pasteur effect. Thus, the in situ enzyme activity is a function of intracellular topography and reciprocal interactions with other pathways. (Author Abstract)

IV. METABOLIC AND BIOCHEMICAL STUDIES WITH MYCOTOXINS

A. Activation and Metabolism of Mycotoxins

122. METABOLIC ACTIVATION OF MYCOTOXINS BY ANIMALS AND HUMANS: AN OVERVIEW.

Shank RC
Community Environmental Medicine, Medical Pharmacology and Therapeutics, Coll. Medicine, Univ. California, Irvine, CA 92717
J Toxicol Environ Health; 2(6):1229-1244 1977

Comparative studies of the metabolism of aflatoxins in relation to their carcinogenicity are reviewed. Early studies using ^{14}C-ring-labeled or methoxy-labeled aflatoxin B1 (AFB1), the most potent carcinogen of the group, suggested that the ring structures of AFB1 are stable but that the compound is rapidly O-demethylated in the rat. Five hydroxy derivatives or metabolites of AFB1 (AFP1, AFM1, AFB2a, AFQ1, and AFR0) appear to be involved in detoxification pathways. The comparative toxicity, carcinogenicity, and metabolism of AFB1 for 12 species are summarized. Species highly sensitive to liver cancer are characterized by a slow rate of AFB1 metabolism at high doses. Epoxidation of the 2,3-double bond in the terminal furan ring of AFB1 apparently results in the active metabolite AFB1 2,3-epoxide. This epoxide decomposes to form a carbonium ion at C-2 that attacks neutrophilic sites in DNA, especially on the guanine moiety. Thus, AFB1 appears to be activated to an alkylating agent in a manner similar to that of carcinogenic N-nitroso compounds and polycyclic hydrocarbons. (75 Refs)

123. THE METABOLIC ACTIVATION OF CHEMICAL CARCINOGENS - RECENT RESULTS WITH AROMATIC AMINES, SAFROLE AND AFLATOXIN B1. (PP. 153-180)

Miller JA, Miller EC
Screening Tests in Chemical Carcinogenesis. Edited by R Montesano, H Bartsch, L Tomatis, W Davis
International Agency for Res on Cancer Brussels Belgium 9-12 June 1975, no. 12, 1976.

Most chemical carcinogens are precarcinogens that must be converted in vivo to ultimate carcinogens. These ultimate carcinogenic forms are strong electrophilic reactants and consequently interact covalently with macromolecules in target tissues in vivo. These general principles have been illustrated by studies of the metabolic activation of aromatic amines and amides, safrole, and aflatoxin B1, which are discussed briefly. The ultimate carcinogenic forms of these substances are believed to be electrophilic metabolites of N-hydroxy derivatives of aromatic amines and amides; the aflatoxin metabolite, aflatoxin B1 -2,3-oxide; and the electrophilic molecular species formed via the esterification of the safrole metabolite, 1'-hydroxysafrole, or the electrophilic molecular species generated via other routes of the oxidative metabolism of safrole. The generalization that ultimate chemical carcinogens possess strong electrophilic properties provides a sound theoretical basis for the use of tissue-mediated mutagenicity assays as rapid screens to detect chemicals, either as pure compounds or in mixtures, that may pose carcinogenic hazards for man. This follows from the fact that the active forms of most mutagens, with the exception of the base analog and simple frameshift mutagens, are also strong electrophilic reactants.

124. AFLATOXIN B1-2,3-OXIDE: EVIDENCE FOR ITS FORMATION IN RAT LIVER IN VIVO AND BY HUMAN LIVER MICROSOMES IN VITRO.

Swenson DH, Miller EC, Miller JA
U. Wisconsin Med. Ctr., Madison
Biochem Biophys Res Commun; 60(3):1036-1043 1974

Four male Fischer rats were injected ip with 3H-aflatoxin B1 (AFB1) (72 microg) in dimethylsulfoxide. In each case, the liver DNA, rRNA, and protein were isolated 14 hr later. **Nine percent of the 3H injected as 3H-AFB1 was recovered covalently bound to the liver macromolecules.** The specific activities of the DNA and rRNA were 15 and 20 times, resp, those of the protein. Mild hydrolysis of the DNA and rRNA adducts yielded a product indistinguishable from 2,3-dihydro-2,3-dihydroxy-aflatoxin B1. The data indicate that approximately 60% of the nucleic acid adducts were derived from in vivo reactions between the metabolically generated electrophile AFB1-2,3-oxide and nucleophilic nitrogen and oxygen atoms in the DNA and rRNA. The acid hydrolysis of rRNA-3H-AFB1 adduct formed by human liver microsomes (obtained from a 48-yr-old female suspected of having Hodgkin's disease) in vitro also liberated the dihydrodiol in significant quantities. The 2,3-oxide of AFB1 is a probable ultimate carcinogenic metabolite.

125. THE METABOLISM OF CHEMICAL CARCINOGENS TO REACTIVE ELECTROPHILES AND THEIR POSSIBLE MECHANISMS OF ACTION IN CARCINOGENESIS. (PP. 737-762)

Miller EC, Miller JA
Chemical Carcinogens, American Chemical Society Monograph 173. Searle CE, ed. Washington DC, American Chemical Society, 1976.

Current data and concepts on the active forms of chemical carcinogens and their reactions with cellular constituents in the induction of neoplasia are reviewed. N-Hydroxy metabolites have been strongly implicated as proximate carcinogenic derivatives of aromatic amines and amides. In a variety of studies, N-hydroxy metabolites generally proved more carcinogenic than the parent compounds. The sulfuric acid ester of N-hydroxy-2-fluorenylacetamide is a major ultimate carcinogenic metabolite in the rat liver, but other ultimate electrophilic derivatives are also probably involved in the carcinogenicity of this compound. The mutagenicity of the nitroso compounds related to a number of carcinogenic aromatic amines and amides, especially 2-nitrosofluorene, suggests the possible importance of these compounds in carcinogenesis. In the case of polycyclic aromatic hydrocarbons, present data indicate that the precursors of DNA-bound hydrocarbon products formed in vivo are epoxides of dihydrodiols and not K-region epoxides, as had been previously thought. The ultimate carcinogenic metabolites of safrole (4-allyl-2-methylenedioxybenzene) include 1'-hydroxysafrole, 1'-oxosafrole, and the 2', 3'-epoxides of safrole, 1'-hydroxysafrole, and 1'-oxosafrole. **The major metabolic precursor of the DNA- and RNA-bound derivatives in the livers of rodents administered aflatoxin B1 is its 2,3-oxide,** which appears to be very labile and, thus, could not be isolated after presumed synthesis by chemical or enzymatic procedures. Both genetic and epigenetic mechanisms of chemical carcinogenesis must be considered, and each may be predominant in specific cases. (211 Refs)

126. COMPARATIVE IN VITRO METABOLISM OF AFLATOXICOL BY LIVER PREPARATIONS FROM ANIMALS AND HUMANS.

Salhab AS, Edwards GS
Dept of Pharmacology, The George Washington Univ Med Center, Washington, D. C. 20037
Cancer Res; 37(4):1016-1021 1977

The metabolism of (^{14}C)aflatoxicol by liver postmitochondrial and microsomal fractions from humans and eight other species was compared. A major metabolic pathway involves the dehydrogenation of aflatoxicol yielding aflatoxin B1. Human liver preparations were more active in this regard than preparations from any of the other species tested. The aflatoxicol dehydrogenase activity was mainly associated with the microsomal fraction and required a hydrogen acceptor (eg, nicotinamide adenine dinucleotide phosphate), but was not inhibited by carbon monoxide, which implies that it was not dependent on the heme-containing microsomal drug-metabolizing system. It had a pH optimum of 8.0. Postmitochondrial liver fractions also oxidized aflatoxicol (and/or the aflatoxin B1 made from it) to at least five other metabolites that comigrated on thin-layer chromatography plates with authentic standards of aflatoxins Q1, P1, H1, M1, and B2 suba. None of these oxidative metabolites were formed in the presence of carbon monoxide. We also report on the in vitro reduction of aflatoxin B1 to aflatoxicol by the cytosol fractions from eight species. Most active in this regard were rabbit and trout preparations, while this activity was almost absent in the guinea pig. Preparations from humans and four other species were intermediate between these extremes. (21 Refs) (Author Abstract)

127. PRELIMINARY STUDY OF IN VITRO AFLATOXIN B1 METABOLISM BY HUMAN LIVER.

Merrill AH, Campbell TC
Dept. Biochem. Nutrition, Virginia Polytechnic Inst. St. U., Blacksburg
Toxicol Appl Pharmacol; 27(1):210-213 1974

(^{14}C)aflatoxin B1 (ring labeled) was incubated for 6.5 hr with 9000 g supernatant of human liver homogenate. The liver tissue was obtained at autopsy from a patient with a brain tumor and at biopsy from a patient with Hodgkin's disease. The chloroform extractable metabolites were quantitated by liquid scintillation counting following purification and identification by thin layer chromatography. The biopsy sample yielded, as percent of the initial concentration: aflatoxin B1, 15.4%; aflatoxin M1, 0.6%; and aflatoxin P1 (free phenol), 1.3%. The autopsy sample yielded: aflatoxin B1, 11.9%; aflatoxin M1, 1.1%; aflatoxin P1 (free phenol), undetected; and two aflatoxin metabolites less polar than aflatoxin B1, 0.9% and 1.7%. Thus, human liver appears to metabolize aflatoxin B1 in a manner similar to that observed with experimental animals. However, in both animals and humans, a significant portion of aflatoxin B1 metabolism remains unknown.

128. AFLATOXIN Q1: A MAJOR METABOLITE OF AFLATOXIN B1 PRODUCED BY HUMAN LIVER.

Buchi GH, Muller PM, Roebuck BD, Wogan GN
Dept. Chem., Massachusetts Inst. Technol., Cambridge
Res Commun Chem Pathol Pharmacol, 8(4):585-592 1974

Aflatoxin Q1, a compound whose structure had not previously been established, represents a quantitatively important metabolic derivative of aflatoxin B1 produced in vitro by human and monkey liver. The 900xg supernatant of a fresh human liver sample converted B1 to Q1 with an efficiency of about 30% in 30 min. Preparative-scale incubations of human and monkey liver preparations resulted in the isolation of a sufficient quantity (23 mg) of Q1 for structure elucidation. It was established that Q1 is a monohydroxylated derivative of B1, with the hydroxyl group on the carbon atom in the position beta to the carbonyl of the cyclopentenone ring.

129. AFLATOXIN Q1: A NEWLY IDENTIFIED MAJOR METABOLITE OF AFLATOXIN B1 IN MONKEY LIVER.

Masri MS, Haddon WF, Lundin RE, Hsieh DP
Western Regional Res. Lab., USDA, Berkeley, Calif.
J Agric Food Chem; 22(3):512-515 1974

The conversion of aflatoxin B1 by rat and monkey liver postmitochondrial preparations to two main metabolites, aflatoxin M1 and aflatoxin Q1, has been reported. In this paper, a study of the structure of aflatoxin Q1 was undertaken. Evidence suggests that aflatoxin Q1 is an isomer of aflatoxin M1 with the hydroxyl group on the carbon atom beta to the carbonyl of the cyclopentenone ring. Aflatoxin Q1 gave a crystalline O-acetyl derivative with acetate anhydride in pyridine. UV spectrum of aflatoxin Q1 had lambda max 366, 267, and 223 nm. IR spectrum was similar to aflatoxin B1 but had absorption bands at 3460 and 3550 cm-1. Mass spectrum of aflatoxin Q1 indicated a molecular weight of 328 and an elemental composition of C17H12O7. The 100-MHz nmr spectrum of aflatoxin Q1 in dimethylformamide-d-7 was similar to that of aflatoxin B1 except for signals assigned to protons of the cyclopentenone ring. In liver postmitochondrial fractions of both monkey and rats, the conversion of aflatoxin B1 to M1 was about 1-3% of the substrate. The conversion to aflatoxin Q1 in the rat liver was also about 1-3%, but was much higher, from 19-52%, in the monkey liver, indicating that aflatoxin Q1 is distinctly a major metabolite in these primates in vitro and may also be a major metabolite in other primates, including man.

130. USE OF MONKEY LIVER MICROSOMES IN PRODUCTION OF AFLATOXIN Q1.

Hsieh DP, Dalezios JI, Krieger RI, Masri MS, Haddon WF
Dept. Environ. Toxicol., U. California, Davis
J Agric Food Chem; 22(3):515-517 1974

Aflatoxin Q1, a newly identified monkey liver metabolite of aflatoxin B1, was prepared in milligram quantities in the crystalline form of biotransformation of aflatoxin B1 using monkey liver microsomal preparations. Parameters needed for quantitation of aflatoxin Q1 using a spectrodensitometer are determined with the aid of radiolabeled metabolites. An amount as small as 1 ng can be measured with certainty. The availability of aflatoxin Q1 will make possible studies on its chemical properties, toxicity, and possible role in the carcinogenicity of aflatoxin B1.

131. AFLATOXIN B1 HYDROXYLATION BY HEPATIC MICROSOMAL PREPARATIONS FROM THE RHESUS MONKEY.

Krieger RI, Salhab AS, Dalezios JI, Hsieh DP
Dep. Environ. Toxicol., Univ. California, Davis
Food Cosmet Toxicol; 13(2):211-219 1975

The basic biochemical properties of the monkey-liver enzyme systems that catalyze the hydroxylation of aflatoxin B1 to aflatoxins M1 and Q1 are reported. Cell free liver preparations from rhesus monkeys were fortified with NADPH and incubated with labeled aflatoxin B1. Aflatoxin Q1 was the primary metabolite, with smaller amounts of aflatoxin M1 and three unidentified organosoluble metabolites also formed. The enzyme systems involved were localized in the microsomal fraction and required NADPH and molecular oxygen for maximum catalytic activity. Both hydroxylase systems were inhibited by carbon monoxide and by SKF 525-A . On the basis of these biochemical properties, the aflatoxin B1 hydroxylases of monkey liver can be classified as typical mixed-function oxidases. It is suggested that since aflatoxin B1 is rapidly metabolized to the less toxic aflatoxin Q1 by monkeys, they may be more susceptible to its acute toxicity and less vulnerable to its chronic or carcinogenic effects than animals which metabolize the compound more slowly. Similarities of aflatoxin metabolism in man and monkeys were discussed.

132. GENETICS OF AFLATOXIN B1 (AFB1) METABOLISM IN VARIOUS INBRED STRAINS OF MICE, OFFSPRINGS OF SELECTED MATINGS AND IN RECOMBINANT INBRED SUBLINES. (MEETING ABSTRACT).

Gurtoo HL, Dahms R, Motycka L, Taylor B
Grace Cancer Drug Center, Roswell Park Memorial Inst, Buffalo, NY 14263
Fed Proc; 36(3):939 1977

Aflatoxin M1 (AFM1, 4-hydroxy AFB1) and DNA-alkylating metabolite, presumably 2,3-AFB1 oxide, are among several hepatic microsome-mediated metabolites of AFB1. Formation of AFB1-oxide appears to be catalyzed by a cytochrome P-450 mediated system. It is inducible by phenobarbital (PB) but not by 3-methylcholanthrene (MC) in mice of C57Bl/6, C57L or AKR strains, or among C57L X AKR recombinant inbred lines. In contrast, formation of AFM1 appears to be catalyzed by a cytochrome P-448 mediated system. It is inducible by MC as well as by PB. Among 15 inbred mouse strains, the C57L X AKR and C57Bl/6 X DBA/2 recombinant inbred lines, and the C57Bl/6 X DBA/2 backcross and F-2 progeny, MC inducibility of AFM1 formation cosegregated with aryl hydrocarbon hydroxylase (AHH) inducibility. We conclude that for AFB1, the formation of DNA-alkylating activity is catalyzed by a cytochrome P-450 enzyme system that is distinct from the cytochrome P-448 AFM1 forming system. The inducibility of AFM1 formation appears to be expressed as a simple autosomal trait determined by a single gene in C57Bl/6 and DBA/2 mouse strains. (Author Abstract)

133. SPECIES COMPARISON OF IN VITRO METABOLISM OF AFLATOXIN B1.

Roebuck BD, Wogan GN
Dept of Pathology, Dartmouth Medical Sch, Hanover, NH 03755
Cancer Res; 37(6):1649-1656 1977

The metabolism of (^{14}C)aflatoxin B1 by 9000 X g supernatant fraction of livers of duck, rat, mouse, monkey, and humans was compared by incubating the compound and liver fractions in the presence of cofactors and air for 30 min. The incubation medium was extracted with chloroform, and the soluble metabolites were separated on thin-layer plates and quantified; radioactivity remaining in the aqueous phase was determined in order to quantify metabolism to water-soluble derivatives. Duck, monkey, and human livers were most active in total conversion, each metabolizing approximately 80% of available substrate in 30 min. Rat and mouse livers had lower activities, metabolizing 15 to 20%. Duck liver produced mainly (60%) chloroform-insoluble derivatives, but all other species produced larger quantities of chloroform-soluble than insoluble metabolites. Aflatoxin Q1 was the principal chloroform-soluble metabolite produced by monkey, human, and rat liver, whereas duck liver produced mainly aflatoxicol in that fraction. Aflatoxin P1 was produced by monkey, human, and mouse liver, but not by duck and rat. The chromatographic region containing M1 and B2a contained low levels of radioactivity in all species except human. No consistent pattern of metabolism emerged which could be correlated with species differences in response to aflatoxin B1 toxicity or carcinogenicity. (27 Refs) (Author Abstract)

134. IN VITRO METABOLISM OF AFLATOXINS B1 AND B2: SPECIES COMPARISON.

Roebuck BD, Siegel WG

Massachusetts Institute of Technology, Cambridge, Mass. 02139

Proc Am Assoc Cancer Res; 16:94 1975

Metabolism of aflatoxins B1 and B2 (AFB1,AFB2) by 9000 x g supernatant of duck, rat, mouse and human livers was studied to compare toxic properties with metabolism. Ring-labeled ^{14}C-AFB1 and AFB2 were incubated in air for 30 min at 37 degrees C with 9000 x g supernatant in a medium optimized for microsomal enzyme activity. Following chloroform extraction, radioactive products were quantified in both phases and the organic soluble aflatoxins were separated, identified, and quantitatively determined on TLC plates. Large species differences in metabolic capacity were found. The rat and mouse both metabolized less than 15% of the AFB1 in 30 min, whereas they metabolized AFB2 even less actively (less than 6%). Human livers showed widely varying capacities for AFB1 metabolism. In part, this may be attributable to clinical exposure to microsomal enzyme inducing agents. The same human liver preparations had little activity for AFB2 metabolism (less than 6%) in 30 min. Duck was the only species which rapidly metabolized both aflatoxins. From AFB2, the duck produced significant quantities (up to 7%) of AFB1. This transformation of AFB2 into B1 may account for the similarity in LD50 values of these two hepatotoxic agents in the duck (Wogan et al, Cancer Res. 31:1936, 1971). The rat and mouse did not produce AFB1 from B2 nor did the humans investigated. The LD50 values of B1 and B2 in the rat differ by 200-fold. (Author Abstract)

135. AFLATOXICOL AS A POSSIBLE PREDICTOR FOR SPECIES SENSITIVITY TO AFLATOXIN B1 (AFB1).

Edwards GS, Rintel TD, Parker CM

Dept. of Pharmacology, George Washington University Medical Center, Washington D.C. 20037

Proc Am Assoc Cancer Res; 16:133 1975

We have examined the ability of liver post-mitochondrial supernatant fractions to metabolize aflatoxin B1 in vitro. Species studied included mouse, rat, cat, dog, monkey, rabbit, guinea pig, duckling, trout, hamster and human. A correlation exists between the ability of these preparations to produce aflatoxicol (aflatoxin Ro, AFRo) from AFB1 and the sensitivity of the species to the acute and carcinogenic effects of AFB1. Several of these species also make a second metabolite (as yet unidentified) which is separated from AFTo only with some difficulty. Both metabolites are made by heat-sensitive, carbon monoxide-insensitive enzymes present in the 105,000 x g supernatant fraction. AFRo production is unaffected by holding the post-mortem liver for up to 8 hours at room temperature or by a single freeze-thaw cycle. The major oxidative metabolites of AFB1 (P1, M2, B2a) showed no correlation to species sensitivity. Humans were much less variable in the production of AFRo than of oxidative metabolites. On basis of the ability of liver preparations to produce AFRo, we estimate humans to be of intermediate sensitivity to AFB1 similar to hamsters and monkeys. (Author Abstract)

136. METABOLISM OF AFLATOXIN B1 TO AFLATOXINS Q1, M1 AND P1 BY MOUSE AND RAT.

Dahms R, Gurtoo HL

Department of Experimental Therapeutics and Grace Cancer Drug Center Roswell Park Memorial Institute New York State Department of Health Buffalo, New York 14263

Res Commun Chem Pathol Pharmacol; 15(1):11-20 1976

We have used a microtechnique to study (a) the metabolism of aflatoxin (AF)B1 by rat and mouse, and (b) the effect of phenobarbital treatment in vivo on the in vitro metabolism of AFB1 by hepatic microsomes from rat and mouse. The results indicate that AFP1, the omicron-demethylated product of AFB1, is a major metabolite produced by the mouse. Although it is detectable in rat, the amount produced is negligible and was calculated to be at least 10 times less than that produced by the mouse. Using several microincubations, AFP1 was prepared in sufficient quantities to verify its identity by UV spectroscopy and by thin layer chromatography against an authentic standard in six different solvent systems. Phenobarbital pretreatment resulted in an enhancement in the total metabolism of AFB1, as well as in the formation of AFM1, AFQ1 and AFP1. (Author Abstract)

137. COMPARATIVE METABOLIC CONVERSION OF AFLATOXIN B1 TO M1 AND Q1 BY MONKEY, RAT AND CHICKEN LIVER.

Masri MS, Booth AN, Hsieh DP

West. Regional Res. Lab., Agr. Serv., Berkeley, Calif.

Lif Sci; 15(2):203-212 1974

The in vitro metabolism of aflatoxin B1 by liver microsomal preparations from monkey (three species), rat, and chicken was studied. With all species, both the previously recognized metabolite aflatoxin M1 and the newly identified aflatoxin Q1 were produced from the aflatoxin B1 substrate. Aflatoxin Q1 is an isomer of aflatoxin M1 with the hydroxyl on the carbon to the carbonyl of the cyclopentenone ring; it was recently discovered following the incubation of rat and monkey liver preparations with aflatoxin B1. These incubations did not produce detectable aflatoxin P1, which has been reported as a major metabolite of aflatoxin B1 in living monkeys. In general, the conversion to aflatoxin M1 was comparable among the different species (1-3% of the substrate), except in the chicken in which it was lower (0.0-0.3%). The conversion to Q1 was comparable to or slightly higher than the conversion to M1 in the rat and chicken liver preparations, but the conversion of Q1 in the monkey liver preparations was significantly higher, accounting for 19-52% of the substrate in three species of monkeys tested.

138. THE FATE OF OCHRATOXIN A IN RATS.

Chang FC, Chu FS

Food Res. Inst., Univ. Wisconsin, Madison, WI 53706

Food Cosmet Toxicol; 15(3):199-204 1977

Toxicologic studies were conducted on rats receiving single ip injections of 1 mg of ^{14}C-labeled ochratoxin (OA). OA reached its highest levels in the serum (equivalent to approx 90% of the dose), liver (4.5%), and kidney (4.4%) 30 min after injection and then decreased gradually, although at 24 hr the samples still contained up to 1 ug OA/g. Electrophoresis of rat serum demonstrated that OA was predominantly bound to the serum albumin fraction in vivo. The toxin was excreted primarily via the urine (over 50% during the first 24 hr) either in its original form (approx 45%) or as unidentified metabolites. Only 13% of the injected OA was excreted in the feces within the first 24 hr, and 77% of this was unaltered OA. On the basis of these data, it is recommended that the meat and organs of animals suffering from mycotoxic nephropathy be rejected for food use because of possible contamination with OA or other mycotoxins. (23 Refs)

139. EFFECT OF SEX DIFFERENCE ON THE IN VITRO AND IN VIVO METABOLISM OF AFLATOXIN B1 BY THE RAT.

Gurtoo HL, Motycka L

Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, New York 14263

Cancer Res; 36(12):4663-4671 1976

Hepatic microsome-catalyzed metabolism of aflatoxin B1 (AFB1) to aflatoxin M1 and aflatoxin Q1 and the 'metabolic activation' of AFB1 to DNA-alkylating metabolite(s) were studied in normal male and female Sprague-Dawley rats, in gonadectomized animals, and in castrated males and normal females treated with testosterone. Microsomes from male animals formed 2 to 5 times more aflatoxin M1, aflatoxin Q1, and DNA-alkylating metabolite(s) than those from females. Castration reduced the metabolism of AFB1 by the microsomes from males by about 50%, whereas ovariectomy had no significant effect on AFB1 metabolism by the microsomes from females. Testosterone treatment (4 mg/rat, 3 times/week for about 6 weeks) of castrated immature males and immature females enhanced the metabolism of AFB1 by their microsomes. A sex difference in the metabolism of AFB1 by liver microsomes was also seen in other strains of rats tested: Wistar, Long-Evans, and Fischer. The activity of kidney microsomes for metabolic activation was 1 to 4% that of the liver activity and was generally lower in microsomes from male rats as compared to those from female rats of Sprague-Dawley, Wistar, and Long-Evans strains. The in vitro results obtained with hepatic microsomes correlated well with the in vivo metabolism of AFB1, in that more AFB1 became bound in vivo to hepatic DNA isolated from male rats and from a female rat treated with testosterone than that isolated from control female rats. These data suggest that the differences in hepatic AFB1 metabolism may be the underlying cause of the sex difference in toxicity and carcinogenicity of AFB1 observed in rats. (Author Abstract)

140. METABOLISM OF AFLATOXIN B1 AND ITS METABOLISM-DEPENDENT AND INDEPENDENT BINDING TO RAT HEPATIC MICROSOMES.

Gurtoo HL, Campbell TC
Roswell Park Mem. Inst., Buffalo, N. Y.
Mol Pharmacol; 10(5):776-789 1974

The metabolism of aflatoxin B1 by rat hepatic microsomes was studied with difference spectroscopy. Microsomal preparations were purified from normal or phenobarbital-stimulated male Sprague-Dawley rats and were incubated with aflatoxin in the presence of an NADPH-generating system. Aflatoxin metabolites were isolated by Sephadex gel filtration. A decrease in absorbance at 360 nm due to the disappearance of aflatoxin B1 during its metabolism, was caused by the formation of a metabolite having a spectral peak at 398 nm. From the solubility, spectral and microsomal binding characteristics, this metabolite appears to be aflatoxin B2a (aflatoxin hemiacetal). Difference spectroscopic studies on microsomes reisolated from incubation mixtures containing aflatoxin B1 showed that aflatoxin B1 was bound to microsomes, giving a complex having a spectral peak at 360 nm; this binding did not require NADPH and the complex could be separated by gel filtration chromatography. A metabolite of aflatoxin B1, the formation of which was NADPH-dependent, was bound to microsomes, yielding a complex having a spectral peak at 405 nm; this complex was not dissociated by gel chromatography or by treatment with trichloracetic acid or extraction with chloroform and acetone. SKF 525-A and L-cysteine inhibited the formation of this complex, the former by inhibiting the metabolic conversion of aflatoxin B1 to aflatoxin B2a and the latter by blocking the binding of the metabolite to microsomes. Aflatoxin B1 and aflatoxin B2a appeared to bind to different sites on the microsomes. A change in pH caused an alteration in the structure of aflatoxin B2a and its extraction with chloroform. pH equilibria for this phenomenon were estimated to be 3.00 and 7.35. Based on these studies, it is proposed that aflatoxin B2a, under alkaline conditions, cleaves to yield a dialdehyde derivative(s) which binds to microsomes, forming Schiff bases with free amino groups. These studies also indicate that under certain conditions, rat hepatic microsome-mediated conversion of aflatoxin B1 to a water-soluble metabolite, which appears to be aflatoxin B2a, represents a major metabolic pathway.

141. OXIDATIVE METABOLISM OF AFLATOXIN B1 BY RAT LIVER MICROSOMES IN VITRO AND ITS EFFECT ON LIPID PEROXIDATION.

Raj HG, Santhanam K, Gupta RP, Venkitasubramanian TA
Vallabhbhai Patel Chest Inst., U. Delhi, India
Res Commun Chem Pathol Pharmacol; 8(4):703-706 1974

Spectral interaction of aflatoxin B1 with phenobarbital-induced rat liver microsomes and the inhibition of aflatoxin B1 mixed function oxidase by SKF 525-A revealed the participation of cytochrome P-450 in the oxidative metabolism of the toxin. EDTA-Fe complex, which generally enhances aromatic hydroxylations, was found to be inhibitory to the aflatoxin B1 oxidative metabolism. Microsomal lipid peroxidation was inhibited in the presence of metabolizing aflatoxin B1 and the inhibition was reversed by SKF 525-A.

142. AFLATOXIN B1 METABOLISM TO AFLATOXICOL AND DERIVATIVES LETHAL TO BACILLUS SUBTILIS GSY 1057 BY RAINBOW TROUT (SALMO GAIRDNERI) LIVER.

Schoenhard GL, Lee DJ, Howell SE, Pawlowski NE, Libbey LM, Sinnhuber RO
Dept. Drug Metabolism, Searle Labs., Box 5110, Chicago, IL 60680
Cancer Res; 36(6):2040-2045 1976

The conditions for the formation, isolation, and identification of aflatoxicol after incubation of aflatoxin B1 with liver homogenates from Mt. Shasta rainbow trout (salmo gairdueri) were determined; the microbial lethality of aflatoxin with and without activation was also tested. The structure (confirmed by mass IR, and UV spectrometry) was identical to one of the diastereomers prepared by chemical reduction of aflatoxin B1 . Aflatoxicol was apparently formed by a NADPH-dependent soluble enzyme of the 105,000 x g supernatant from rainbow trout. Neither aflatoxicol nor aflatoxins B1 , Q1 , and B2 were lethal in phosphate buffer to Bacillus subtilis GSY 1057 (metB4, hisA1, uvr-1). In the presence of NADPH and trout liver microsomes, aflatoxical reduced the viability of B subtilis. Aflatoxin B2 , which lacks the vinyl ether present in the other compounds, could not be activated. The product of aflatoxin B1 activation by trout liver microsomes was sought after incubation of ^{14} C-labeled aflatoxin B1 . The radioactivity was found in unaltered aflatoxin B1 and in three extremely polar metabolites. The quantity of the new metabolites and the level of microbial lethality was reduced by addition of cytosine and cysteine to the incubation medium. The vinyl ether configuration was a structural requirement for activation. This finding and the nature of the enzymatic reaction was consistent with the hypothesis that the compounds were metabolized to highly reactive and unstable electrophilic products that bound to nucleophiles such as cytosine and were lethal to B subtilis.

143. FORMATION OF AFLATOXIN B1 FROM AFLATOXICOL BY RAINBOW TROUT (SALMO GAIRDNERI) LIVER IN VITRO.

Loveland PM, Sinnhuber RO, Berggren KE, Libbey LM, Nixon JE, Pawlowski NE
Department of Food Science and Technology, Oregon State University Corvallis, Oregon 97331 ·
Res Commun Chem Pathol Pharmacol; 16(1):167-170 1977

Aflatoxicol (R subo) is produced from the in vitro incubation of aflatoxin B1 with submitochondrial liver fractions from several species. In chickens, ducks, turkeys, and rabbits, aflatoxicol is the major metabolite from incubation of B1 with the soluble fraction (Patterson and Roberts, 1971). The primate reductase enzyme system also produces R subo (Salhab and Hsieh, 1975). In the rainbow trout, the animal studied in our laboratory, R subo is produced as the major metabolite by the soluble fraction enzymes of the liver (Schoenhard, et al, 1976). On the other hand, R subo has apparently not been detected in the mouse, rat or guinea pig (Patterson and Roberts, 1972). The enzyme considered to be responsible for the reduction of B1 to R subo is the soluble, NADPH-dependent 17-hydroxy-steroid dehydrogenase (Campbell and Hayes, 1976). In the case of duck liver, Patterson and Roberts (1972) concluded from their kinetic data that the R subo produced from B1 was being converted back to B1. Aflatoxin B1 is toxic and carcinogenic in varying degrees to these animals. Likewise R subo has been shown to be toxic and carcinogenic, but to a lesser degree than B1, in rainbow trout (Schoenhard, 1974). We undertook the present study to determine if R subo, the major in vitro B1 metabolite produced by the submitochondrial fraction of rainbow trout liver, could be converted back to B1 in the same system. (Author Abstract)

144. AFLATOXICOL M1, A NEW METABOLITE OF AFLATOXICOL.

Salhab AS, Abramson FP, Geelhoed GW, Edwards GS
Dept. Pharmacology, George Washington Univ. Medical Sch., 2300 Eye St., NW, Washington, DC 20037
Xenobiotica; 7(7):401-408 1977

A new metabolite of aflatoxicol, aflatoxicol M1, was isolated from the postmitochondrial and microsomal fractions of dog liver fortified with NADPH. This metabolic transformation is similar to the conversion of aflatoxin B1 to aflatoxin M1. Aflatoxicol M1 can also be produced from aflatoxin M1 by a reductase present in rabbit liver cytosol, a reaction analogous to the reduction of aflatoxin B1 to aflatoxicol. In addition, aflatoxicol M1 can be oxidized to aflatoxin M1 by a carbon monoxide-insensitive dehydrogenase activity associated with human liver microsomes, as in the production of aflatoxin B1 from aflatoxicol by this fraction. The UV, fluorescence, and mass spectral characteristics of aflatoxicol M1 are described. The possibility that this new aflatoxin metabolite is both toxic and carcinogenic is discussed. (14 Refs)

145. MYCOTOXINS: METABOLISM AND LIVER INJURY.

Patterson DS
Central Vet. Lab., Weybridge, Surrey, U. K.
Biochem Soc Trans; 1(4):917-922 1973

The metabolic fates of the aflatoxins, sterigmatocystin, ochratoxins A and B, and the trichothecene series of mycotoxins are discussed. Aflatoxin B1 is probably converted to a highly reactive metabolite before causing liver injury. Generally, the rate of metabolism is fastest in species that are highly susceptible to acute aflatoxin poisoning. These species convert aflatoxin B1 quickly into the 2-hydroxy derivative, the hemiacetal. The hemiacetal binds to proteins, probably forming Schiff bases and possibly causing liver necrosis in a nonspecific manner. Aflatoxin G1 can also be converted into its hemiacetal in the liver. However, aflatoxins B2 and G2 cannot undergo this transformation and are correspondingly less toxic. It is also possible that the carcinogenic properties of aflatoxin may be dependent on its conversion into a 2,3-epoxide, which may exist as a transient intermediate in the liver. The structure of sterigmatocystin is similar to that of aflatoxin, and it can be converted into its hemiacetal by treatment with mineral acid. However, there is no evidence that this transformation takes place in the liver. Ochratoxin A is metabolized slowly in the liver, and there is evidence that its phenolic hydroxyl group is important to its hepatotoxicity. Scirpene triacetate is detoxified by the liver. Unstimulated metabolism does not primarily involve epoxide hydration, whereas phenobarbital pretreatment stimulates this mode of detoxification.

146. THE ROLE OF AFLATOXIN METABOLISM IN ITS TOXIC LESION.

Campbell TC, Hayes JR
Div. Nutritional Sciences, Cornell Univ., Ithaca, NY 14853
Toxicol Appl Pharmacol; 35(2):199-222 1976

The public health significance and fundamental mechanism of action of aflatoxin, which has been extensively investigated since its discovery over 15 yr ago, were reviewed. The substance is composed of a group of bis-furanocoumarins, which can be metabolized by the drug-metabolizing enzyme system in addition to other enzymes. The concept that microsomal enzyme activation is required before cytotoxicity can be expressed has won wide support. Many aflatoxin B1 metabolites have been identified and their toxicological properties examined. Understanding the complex metabolism of aflatoxin would further elucidate the toxicological significance of the compound as well as identify the critical molecular interaction that initiates the lesion. (132 refs)

147. THE METABOLIC TRANSFORMATION OF THE MYCOTOXIN, AFLATOXIN B1.

Steyn PS
No affiliation given
S Afr J Sci; 73(7):200-201 1977

The various metabolic reactions that could be involved in the breakdown of aflatoxin B1 are outlined, including conversion to aflatoxin M1 and aflatoxicol H1. (17 Refs)

148. IN VITRO METABOLIC CONVERSION OF AFLATOXINS AND BENZO(A)PYRENE TO NUCLEIC ACID-BINDING METABOLITES.

Gurtoo HL, Dave CV
Roswell Park Mem. Inst., Buffalo, N.Y.
Cancer Res; 35(2):382-389 1975

The effects of inducers and inhibitors of microsomal mixed-function oxygenase and the relationship of aflatoxin structure to the generation of the reactive metabolites that bind to DNA and RNA were investigated and the enzymatic pathways for the formation of DNA-binding metabolites from aflatoxin B1 and from benzo(a)pyrene were compared. Hepatic microsomes were isolated from untreated, phenobarbital-treated (40 mg/kg ip), and 3-methylcholanthrene-treated (15 mg/kg, ip) rats. In the standard assay, 3H aflatoxin B1 was incubated with rat hepatic microsomes, rat liver RNA, or native calf thymus DNA in the presence or absence of a nicotinamide adenine dinucleotide phosphate-generating system. An aflatoxin B1 metabolite became covalently bound to rat liver RNA and calf thymus DNA in vitro and formed complexes with increased spectral absorbance in the 360 nm region. The formation of such complexes, which was microsome- and reduced nicotinamide adenine dinucleotide phosphate -dependent, was inhibited by beta-diethyl-aminoethyl diphenylpropylacetate-HCl, and by CO and N2, when the later were used to replace the gas phase of the incubations. The formation of the complexes was enhanced about 2-fold with microsomes from phenobarbital -treated rats but not from 3-methylcholanthrene -treated rats. More binding was observed with DNA than with RNA. In the presence of reduced nicotinamide adenine dinucleotide phosphate and microsomes from phenobarbital-treated rats, aflatoxin G1 was also converted into metabolite(s) that became covalently bound to nucleic acids and formed complexes with increased spectral absorbancies in the 360 nm region. Under the same conditions, aflatoxin B2 , aflatoxin B2a , aflatoxin G2, and 'Compound 11', which lack a C2-C3 double bond, did not show any noticeable binding to either DNA or RNA. These data support the concept that the microsomal mixed-function oxygenase-catalyzed oxidation of the C2-C3 double bond of aflatoxins is a prerequisite for the formation of nucleic acid-binding metabolites. In assays involving the formation of DNA-binding metabolites from benzo(a)pyrene, microsomes from 3-methyl-cholanthrene-treated rats were 12- and 5-fold more active than microsomes from untreated and phenobarbital-treated rats, resp. This suggests that different enzymes in hepatic microsomal mixed-function oxygenase complex are involved in the generation of reactive metabolites from various polycyclic hydrocarbons.

149. HEPATIC MICROSOMAL MIXED FUNCTION OXYGENASE: ENZYME MULTIPLICITY FOR THE METABOLISM OF CARCINOGENS TO DNA-BINDING METABOLITES.

Gurtoo HL, Bejba N
Roswell Park Mem. Inst., Buffalo, N.Y.
Biochem Biophys Res Commun; 61(2):735-742 1974

Microsome-mediated metabolic activation of aflatoxin B1 (AFB1) and benzoapyrene (BP), as determined by the in vitro formation of DNA binding metabolites, was studied, using hepatic microsomes from untreated, phenobarbital (PB)-treated and 3-methylcholanthrene (MC)-treated male Sprague-Dawley rats. In the case of AFB1 , microsomes from PB-treated rats were twice as active as microsomes from untreated and MC-treated rats, whereas, in the case of BP, microsomes from MC-treated rats were several fold more active than microsomes from untreated and PB-treated rats. These data strongly suggest enzyme multiplicity of microsomal mixed function oxygenase for the activation of carcinogens, especially AFB1 and BP whose reactive metabolites are believed to be epoxides.

B. Binding of Mycotoxins to Cellular Components and Macromolecules

150. CARCINOGEN-PROTEIN COMPLEXES IN LIVER CYTOSOL DURING HEPATOCARCINOGENESIS BY AFLATOXIN B1. (MEETING ABSTRACT).

Mainigi KD, Sorof S
The Inst for Cancer Res, Fox Chase Cancer Center,
Philadelphia, PA 19111
Fed Proc; 36(3):349 1977

In search of common types of protein complexes of various carcinogens in their target organs, this study examined the kinds and amounts of carcinogen-protein complexes formed in liver during hepatocarcinogenesis by aflatoxin B1 (AFB1). Adult male CDF rats were continously fed AFB1 (1 ppm) or the control diet up to 24 weeks, then given ^{3}H-AFB1 (50 muCi/100 gm) intragastrically, and fed the control diet for 18 or 48 hr until sacrifice. Liver cytosolic macromolecules, obtained by Sephadex G-25 gel, were resolved into multiple protein components according to charge and size by DEAE Sephadex A-50 and by Sephadex G-200 gel. Profiles of the in vivo-formed complexes were compared throughout the course of the carcinogenesis, and with those formed by in vitro incubation of cold liver cytosols with ^{3}H-AFB1. At least three major and two minor species of complexes, as resolved by A-50, were formed in the rats fed the carcinogen or control diet. More than five major types of complexes were detected after in vitro incubation with ^{3}H-AFB1. Whereas the in vivo-formed complexes exhibited considerable molecular size heterogeneity, in vitro incubation gave rise only to one major (4S) and one minor (15-20S) size species. Relatively very little ^{3}H-AFB1 or its metabolites were bound to ligandin. Only slight change in types and amounts of complexes occurred during the carcinogenesis. (Author Abstract)

151. STUDY ON THE BINDING OF AFLATOXIN B1 TO CELLULAR COMPONENTS (I) BINDING OF AFLATOXIN B1 TO ALBUMIN IN VITRO.

Lu FJ, Ling KH
J Formosan Med Assoc; 72:434-439 1973

The aflatoxin B1-albumin complex is isolated by Sephadex G-25 column chromatography. The molar ratio of the toxin to the protein is 1 to 265. The complex is stable in an alkaline solution of pH 8.6 and overnight dialysis. However, it is completely dissociated in 1 M guanidine hydrochloride solution. It is likely some hydrogen bindings are associated between the toxin and the protein. (Author Abstract)

152. BINDING OF AFLATOXINS B1 AND G1 TO RAT LIVER CHROMATIN.

Prasanna HR, Gupta SR, Viswanathan L,
Venkitasubramanian TA
Dept. Biochemistry, Vallabhbhai Patel Chest Inst., Univ.
Delhi, Delhi 110007, India
Toxicol Appl Pharmacol; 36(3):503-510 1976

Fluorescence quenching was used to measure toxin-chromatin interaction. Interaction of aflatoxin B1 with components of chromatin increased in the following order: nonhistone residue, DNA, histone, histone-free chromatin, and intact chromatin. Fluorescence change due to interaction of aflatoxin B1 with various complexes of chromatin constituents was not significantly different from that obtained with DNA. Association of aflatoxin B1 with chromatin components was very weak, whereas that with reconstituted deoxyribonucleoprotein (consisting of histone, nonhistone residue, and DNA) and with native chromatin was strong, as shown by the increase in fluorescence polarization of aflatoxin. The results suggest the importance of the structural integrity of chromatin in the binding with aflatoxin. Interaction of aflatoxin G1 with chromatin was less than that of aflatoxin B1 . (22 refs)

153. THE EFFECT OF PROTEIN DEFICIENCY ON THE IN VIVO BINDING OF AFLATOXIN B1 TO RAT LIVER MACROMOLECULES.

Preston RS, Hayes JR, Campbell TC
Division of Nutritional Sciences Cornell University Ithaca,
New York 14853
Lif Sci; 19(8):1191-1198 1976

Male, weanling rats divided into three groups were maintained for 15 days on a semipurified diet containing either 5% casein fed ad libitum (group 1), 20% casein pair-fed to group 1 (group 2), or 20% casein fed ad libitum (group 3). Animals on day 16 were injected ip with ^{3}H-AFB1 (1.90 mg/kg) and were sacrificed six hours later. In both the control and protein deficient animals, binding of AFB1 to DNA was greater than that for chromatin protein. In the protein deficient animals, there was a consistent decrease (70%) in binding to chromatin, DNA and chromatin protein. The decrease in binding to nuclear macromolecules in protein deficient animals is correlated with carcinogenicity and mixed function oxidase (MFO) enzyme activity, and the relationships between carcinogenicity, MFO activity, and binding are discussed. (Author Abstract)

154. THE EFFECT OF CYCLOPROPENOID FATTY ACIDS ON BINDING OF AFLATOXIN B1 TO NUCLEAR DNA, RNA AND PROTEIN OF RAT HEPATOCYTES.

Scarpelli DG, Rao MS, Reddy JK
University of Kansas Medical Center, Kansas City, Ks
66103
Proc Am Assoc Cancer Res; 16:164 1975

C18 and C19 fatty acids containing a cyclopropene ring (CPFA's), natural compounds in plants of the order Malvales, have been shown to augment the carcinogenic effects of aflatoxin B1 (AFB1) and were recently identified a potent mitogen for hepatocytes. Experiments to ascertain whether CPFA's affect the binding of AFB1 to DNA, RNA and protein of rat liver cells were carried out. 50 gm male Sprague-Dawley rats were fed a synthetic diet containing 500 ppm of CPFA's supplied as triglycerides in Sterculia foetida oil ad libitum for 2 wks. Comparable animals fed synthetic diet alone served as controls. Animals were injected IP (3 microC/100 gm B.W.) with C^{14} ring labeled chromatographically pure AFB1 (Sp. Ac. 5.37.microC/microM) dissolved in N-methylformamide and sacrificed 1 hr later. DNA, RNA and protein were extracted; DNA was further purified by treatment with pronase, RNA-ase, and by density gradient centrifugation on CsCl2. These results corroborate earlier work (Lijinsky, W., Lee, Y.L., and Gallagher CH. Cancer Res: 30:2280, 1970) that AFB1 binds to a greater degree to protein and RNA than to DNA. Ingestion of CPFA enhanced binding of AFB1 to protein by 39.9%, RNA 35.5% and DNA 37%. This effect of CPFA may be related to its potentiation of AFB1 induced hepatocarcinoma. (Author Abstract)

155. ON THE NATURE OF THE BINDING OF AFLATOXIN B2A TO RAT HEPATIC MICROSOMES.

Gurtoo HL, Dahms R
Roswell Park Mem. Inst., Buffalo, N. Y.
Res Commun Chem Pathol Pharmacol; 9(1):107-118 1974

The in vitro binding of aflatoxin B2a to microsomes isolated from the livers of male Sprague-Dawley rats was studied. Washed microsomes (900 mg protein) were incubated for 40 min with either unlabeled or 3H-aflatoxin B2a (6 mg). The aflatoxin-treated microsomes were isolated by thin-layer chromatography after defatting in organic solvents and removal of unbound protein by protease digestion. Under these conditions, aflatoxin B2a bound to hepatic microsomes, the conjugate having a difference spectral peak in the 405 nm region which was similar to that previously seen for aflatoxin B2a-microsomal conjugates and for unbound aflatoxin B2a. The aflatoxin B2a-microsomal conjugate could not be separated by Sephadex gel filtration or by extraction with several solvents ranging widely in polarity. Less than 10% of the microsome-bound 3H was released by precipitation with 8% trichloroacetic acid, suggesting that binding was probably covalent. The complex was, however, susceptible to protease digestion. Based on these findings the following explanation of binding of aflatoxin B2a to microsomal proteins is offered; aflatoxin B2a cleaves in alkaline medium to form dialdehyde derivatives which form Schiff bases with the free amino groups of microsomal proteins.

156. AFLATOXIN B1-2,3-OXIDE AS A PROBABLE INTERMEDIATE IN THE COVALENT BINDING OF AFLATOXINS B1 AND B2 TO RAT LIVER DNA AND RIBOSOMAL RNA IN VIVO.

Swenson DH, Lin JK, Miller EC, Miller JA
Mc Ardle Laboratory for Cancer Research, University of
Wisconsin Medical School, Madison, Wisconsin 53706
Cancer Res; 37(1):172-181 1977

Administration of (^{3}H)aflatoxin B2 (2,3-dihydroaflatoxin B1) (AFB2) to male rats resulted in levels of hepatic DNA- and ribosomal (r)RNA-aflatoxin adducts that were about 1% of those for rats given (^{3}H)aflatoxin B1 (AFB1). The levels of hepatic protein-aflatoxin adducts were 35 to 70% as great for AFB2-treated as compared to AFB1-treated rats. Mild acid hydrolysis of hepatic DNA and rRNA from rats given (^{3}H)AFB1 or (^{3}H)AFB2 yielded 2,3-dihydro-2,3-dihydroxyaflatoxin B1 and two other major tritiated products that were separable by high-pressure liquid chromatography. With 2-hr hydrolyses approximately 77 and 54% of the ^{3}H from the hepatic DNA of rats given (^{3}H)AFB1 or (^{3}H)AFB2, respectively, and 40 and 32%, respectively, of the ^{3}H from the rRNA of rats given (^{3}H)AFB1 or (^{3}H)AFB2 were isolated as these products. One of the tritiated products was converted to 2,3-dihydro-2,3-dihydroxy-aflatoxin B1 on further mild acid hydrolysis. The high levels of the dihydrodiol and its precursor in the hydrolysates, especially when the 35% loss of added 2,3-dihydro-2,3-dihydroxyaflatoxin B1 under the hydrolysis conditions is taken into account, indicate that aflatoxin B1-2,3-oxide is a major ultimate precursor of the nucleic acid-bound derivatives formed from both AFB1 and AFB2 in rat liver. From these data, AFB2 appears to be converted to AFB1 in the rat liver in vivo to an extent (about 1%) that is comparable to the ratio of hepatocarcinogenicities of these compounds in this species. The levels of hepatic DNA-bound adducts from (^{3}H)AFB1 were about one-half of the control level in hypophysectomized rats, while the levels of the rRNA- and protein-aflatoxin adducts were similar to those of the controls. The levels of the hepatic macromolecule-bound adducts were 10 to 30% of the control levels in phenobarbital-treated rats. The levels of macromolecule-bound adducts of AFB1 in kidney, spleen, and small intestine were much lower than those of liver. The inhibitory action of phenobarbital on AFB1-induced hepatocarcinogenesis was confirmed; administration of benz(a)anthracene or high levels of ascorbic acid with AFB1 did not alter the tumor incidence. (Author Abstract)

157. ELECTROPHILIC AND ULTIMATE CARCINOGENIC DERIVATIVES OF AFLATOXIN B1.

Swenson DH
Univ. Wisconsin-Madison, WI
Diss Abstr Int B; 36(9): 4374B-4375B 1976

The metabolism of aflatoxin B1 to electrophilic derivatives and the roles of such metabolites in carcinogenesis was investigated. The hydrolysis product of **an RNA-aflatoxin B1 adduct** was characterized as 2,3-dihydro-2,3-dihydroxy-aflatoxin B1. The aflatoxin in the adduct was found through its carbon-2 in glycosidic linkages to oxygen or nitrogen atoms in RNA; the reactive intermediate for binding to RNA was aflatoxin B1-2,3-oxide. Similar evidence is presented for the formation of aflatoxin B1-2,3-oxide by human liver microsomes in vitro. Similar fractions of the H3aflatoxin residues in the nucleic acids of the livers, kidneys, spleens, and small intestines of control, phenobarbital-treated, and hypophysectomized rats were released as the dihydrodiol, although the overall amounts of bound metabolites differed widely between the tissues. More than one-half of the nucleic acid-bound aflatoxin formed in vivo apparently resulted from reaction with the 2,3-oxide. Administration of H3aflatoxin B2 gave low levels of hepatic nucleic acid-aflatoxin adducts which yielded the dihydrodiol on hydrolysis. The amount of dihydrodiol released from the nucleic acids generally paralleled the susceptibility of the tissues to carcinogenesis by aflatoxins B1 and B2, except for the liver of hypophysectomized rats. Aflatoxin B1-2,3-dichloride (AFD) was synthesized as a model for this putative metabolite. As expected for the 2,3-oxide, the AFD formed covalent adducts with DNA, ribosomal RNA, and protein. The nucleic acid-bound products retained one-half of the chloride and were apparently bound to the nucleic acids through glycosidic bonds at carbon-

2 of the aflatoxin. AFD was much more active than aflatoxin B1 in inducing tumors at sites of application in rats and mice and was highly mutagenic for bacteria, while aflatoxins B1, B2a, B2 and the dihydrodiol were inactive; the latter four compounds were also inactive as skin tumor initiators. It appears that aflatoxin B1-2,3-oxide is an ultimate carcinogenic metabolite of aflatoxin B1 and that alkylation of nucleic acids is a critical reaction in the induction of tumors with this carcinogen and its 2,3-dichloride. (0 Refs)

158. STUDIES ON AN AFLATOXIN-GUANINE ADDUCT PRODUCED IN VITRO AND IN VIVO IN DNA. (MEETING ABSTRACT).

Essigmann JM, Croy RG, Busby WF, Reinhold VN, Wogan GN
MIT, Cambridge, MA 02139
Proc Am Assoc Cancer Res; 18:179 1977

Aflatoxin B1 (AFB1) was covalently bound to calf thymus DNA in vitro using phenobarbital-induced rat liver microsomes. Optimization of the system resulted in a binding level of one aflatoxin residue per 35 bases. The modified DNA was isolated and depurinated under acidic conditions. Hydrolysates were analyzed by HPLC, and over 90% of the radioactivity incorporated into DNA from ^{3}H-AFB1 was accounted for in a single component which exhibited strong absorptivity at 365 nm but lacked characteristic AFB1 fluorescence. Treatment of adduct with $HClO_4$ liberated guanine, as evidenced by its chromatographic properties, UV-absorbance and mass spectrum. Mass spectral data on the intact adduct showed fragment ions resulting from the guanine and aflatoxin moieties and a molecular ion at m/e 479. A component with identical chromatographic, UV and fluorescence properties was present in DNA isolated from the liver of 150 g rats treated with ^{3}H-AFB1 (0.5 mg/kg) by ip injection. The in vivo product represented over 90% of bound radioactivity released by acidic hydrolysis and accounted for approximately 70% of the material bound covalently to DNA. These results show that an aflatoxin-guanine adduct is the principal product of the interaction of metabolically activated forms of this carcinogen with DNA in vitro and in vivo. (Author Abstract)

159. STRUCTURAL IDENTIFICATION OF THE MAJOR DNA ADDUCT FORMED BY AFLATOXIN B1 IN VITRO.

Essigmann JM, Croy RG, Nadzan AM, Busby WF, Reinhold VN, Buchi G, Wogan GN
Dept. Nutrition and Food Science, Massachusetts Inst. Technology, Cambridge, MA 02139
Proc Natl Acad Sci USA; 74(5):1870-1874 1977

Aflatoxin B1 (AFB1) was activated metabolically by rat liver microsomes and bound to calf thymus DNA in vitro in order to isolate and analyze the structure of the bound form of the carcinogen. The bound intermediate was removed from DNA with formic acid, and 90% of the carcinogen was shown to be a single component by reverse-phase high-pressure liquid chromatography. Larger quantities of the adduct were isolated by high-pressure liquid chromatography, and they were subjected to chemical, nuclear magnetic resonance, UV, and mass spectral analysis. The results showed that the DNA-bound product was 2,3-dihydro-2-(N^7-guanyl)-3-hydroxyaflatoxin B1. Identification was also confirmed by correlating the UV spectra with those of a model compound. The structural data support indirect evidence that aflatoxin B1 2,3-oxide is quantitatively important as an intermediate in the binding of AFB1 to nucleic acids. (36 Refs)

160. THE RELATIONSHIP BETWEEN HEPATIC GLUTATHIONE LEVELS AND THE FORMATION OF AFLATOXIN B1-DNA ADDUCTS AS INFLUENCED BY DIETARY PROTEIN INTAKE (MEETING ABSTRACT).

Allen-Hoffmann BL, Campbell TC
Cornell University, Ithaca, NY 14853
Fed Proc; 36(3):1116 1977

In vivo studies in weanling rats were undertaken to elucidate the effect of dietary protein intake on the covalent binding of AFB1 to DNA as influenced by glutathione (GSH) levels. Weanling rats were employed and were fed ad lib either a 5% casein (I) or 20% casein (II) diet. After 2 wk, AFB1 (5 mg/kg) was injected ip 6 hr prior to sacrifice. Group I animals, prior to dosing, exhibited a 2-3 fold increase in GSH. After AFB1 administration, hepatic GSH levels reached nadirs of 7.0 mM (I) and 1.4 mM (II) 8 hr after dosing. A 200% rebound above initial GSH levels was observed at 26 hr after dosing in (I) but remained suppressed in (II). To deplete hepatic GSH, diethyl maleate (DEM) (0.6 ml/kg) was administered prior to injection of ^{3}H-AFB1. DEM treatment enhanced covalent binding of the reactive AFB1 metabolite to liver DNA in both groups demonstrating that hepatic GSH is inversely related to the formation of AFB1-DNA adducts. Protein deprivation increased GSH levels and depressed AFB1-DNA adducts both in this and earlier experiments from this laboratory (Preston et al, Life Sci 19:1191, 1976). This suggests that protein status in weanling rats influences the binding of carcinogens, which form electrophilic metabolites to DNA by influencing hepatic GSH levels. (Author Abstract)

161. INTERACTION OF AFLATOXIN B1 WITH DNA.

Fujimoto S, Ohba Y
Dep. Biochem., Fac. Sci., Univ. Kanazawa, Japan
J Biochem (Tokyo); 77(1):187-195 1975

The interaction of aflatoxin B1 (A-B1) with native and heat-denatured calf thymus DNA is described. With native DNA, the absorption spectrum of A-B1 underwent a shift in the region of 300-420 nm, with an isobestic point at 376 nm. Alkaline hydrolysis of the DNA-bound A-B1 proceeded more readily than that of the free toxin, and the hydrolyzed product was more labile. Dissociation of the complex was easily accomplished by heat and by increased ionic strength (0.1 M NaCl), and the DNA released from the complex with an isobestic point at 380 nm. Since this complex was stable in 0.1 M NaCl, it is considered that the mechanism of binding is different from that with native DNA. Based on Scatchard plots, it was calculated that one toxin molecule was bound for each 80-140 nucleotide moiety of native DNA. The number of binding sites was doubled in the presence of calf thymus histones. The mechanism of the interaction remains unknown, but presumably intercalation is not involved.

162. MICROSOME-DEPENDENT BINDING OF BENZO(A)PYRENE AND AFLATOXIN B1 TO DNA AND BENZO(A)PYRENE BINDING TO AFLATOXIN-CONJUGATED DNA.

Alexandrov K, Frayssinet C
Inst. Sci. Res. Cancer, Villejuif, France
Cancer Res; 34(12):3289-3295 1974

Aflatoxin B1-conjugated DNA and benzo(a)pyrene (BP)-conjugated DNA were isolated after incubation in vitro with liver microsomes from phenobarbital (PB), methylcholanthrene (MC) and phenobarbital plus methylcholanthrene-treated rats and the NADPH-generating system. A DNA complex with both carcinogens was obtained by fixation of BP to the aflatoxin B1-conjugated DNA. Phenobarbital was injected ip into male Wistar rats (Commentry strain) at 80 mg/kg for three consecutive days. MC (40 mg/kg) was injected ip 48 hr before sacrifice. The microsomes were isolated and incubated with 5 mg DNA and the NADPH-generating system. In two other instances, 80 nM of BP-3H (specific activity, 300 mCi/mM) or 0.5 microM aflatoxin B1-14C (radiochemical purity, 95% and specific activity 1 microCi/micromole) was added to the above. The UV absorption spectra of aflatoxin B1-14C-conjugated DNA was determined from 320-400 nM. The aflatoxin B1-, B2-, B2a-, G1-, G2- and M1-conjugated DNA binding of ethyl acetate-soluble BP3-H metabolites was assayed by radioactivity (39% efficiency for 3-H and 75% for 14C). The number of labeled hydrocarbons/M of DNA-phosphorus was calculated. The BP-3H binding to DNA was maximum with MC and PB + MC microsomes. There was a smaller increase with PB microsomes. Fixation of BP-3H metabolites to DNA without microsomes was similar to that found with MC microsomes. A non-labeled BP-conjugated DNA with 3H-labeled BP metabolites indicated that maximum fixation to specific DNA sites occurred with initial BP metabolite addition. In all experiments with microsomes, absorption for the aflatoxin B1-14C-conjugated DNA was a maximum at 360-370 nm. Radioactivity measurements gave a maximum with the MC microsomes. The number of nucleotides bound by aflatoxin B1 decreased in the presence of all treated microsomes. With PB microsomes, BP and 7,8-benzoflavone (7,8-BF), inhibition of binding was 32% while stimulation of binding was 50% with aflatoxin B1. MC gave 76% inhibition for BP binding and 20% inhibition for aflatoxin B1. Inhibition was 80% for PB + MC and BP and 0% for aflatoxin B1. the results suggest that the binding species of the acetate-soluble BP metabolites are relatively stable. MC activity is more essential to the microsome-dependent binding of BP to DNA than for the binding of aflatoxin B1 to DNA. This is in agreement with previous studies.

163. STRUCTURE ACTIVITY RELATIONSHIP IN THE BINDING OF AFLATOXIN METABOLITES TO DNA (MEETING ABSTRACT).

Gurtoo H, Dave C, Motycka L
Roswell Park Mem. Inst., Buffalo, N.Y.
Proc Am Assoc Cancer Res; 15:42 1974

Covalent binding of an aflatoxin (AF) B1 metabolite to rat liver RNA has been described (Res Commun Chem Pathol Pharmacol 5: 635; 1973). Using spectroscopic techniques, binding of AFB1 metabolite to native calf thymus DNA was studied. The binding was NADPH and microsome dependent and was inhibited by SKF-525A. Microsomes from phenobarbital, but not from 3-methylcholanthrene treated rats, were twice as effective as those from untreated rats. The reaction was also catalyzed by reconstituted cytochrome P-450 system in the presence of NADPH. The extent of binding was similar with native and denatured calf thymus DNA whereas it was greater with native DNA from Mycobacterium lysodeikticus having a high G-C content. AFB2 , AFG2 and "compound 11" showed no binding. AFG1 showed a weak binding which was NADPH and microsome-dependent and also inhibited by SKF-525A. These data strongly support the concept that the oxidation of the C2 -C3 double bond in the aflatoxin moiety is a prerequisite for the formation of DNA alkylating agents. This structural specificity correlates well with that for the carcinogenicity of aflatoxins.

164. DO AFLATOXIN AND POLYCYLIC HYDROCARBON CARCINOGENS DELETE GENES IN THE REGIONS OF CHROMOSOMES ACTED ON BY STEROID HORMONES?

Money-Kyrle AF
39 Gloucester Crescent, London NW1, England
Med Hypotheses; 3(4):146-149 1977

A hypothesis is presented which can account for some of the changes observed in tumor cells following carcinogenesis by aflatoxin B1 and polycyclic hydrocarbons. It is proposed that these carcinogens **bind to regions of DNA** to which steroid hormones bind. The carcinogen then reacts with the guanine bases at these sites; and at cell division, these genes are deleted. This deletion would include genes normally activated or repressed by steroid hormones such as those that bind ribosomes to the endoplasmic reticulum. Thus lysosomal enzymes could be released directly into the cytoplasm and effect changes in contact inhibition, reduce cyclic AMP levels, produce permeability changes, and cause chromosomal abnormalities through DNAses. Experimental evidence supporting this hypothesis is presented, and possible experimental procedures for testing are suggested. (38 Refs)

165. THE BINDING AFFINITY OF AFLATOXINS ON THE UTERINE OESTROGEN RECEPTOR.

Kyrien HJ
Inst. Physiol., Univ. Munich, Germany
Z Lebensm Unters Forsch; 5(154):285-287 1974

The estrogenic potency of the aflatoxins B1, G1, G2 and M1 was determined by incubating the uterine cytosol fraction of calves with tritiated 17beta-estradiol in the presence of the various aflatoxins. If the aflatoxins competed with 17beta-estradiol for binding sites on the receptor, the binding of the 17beta-estradiol was diminished. The aflatoxins B1, G1 and G2 showed no affinity for the uterine estrogen receptor. Aflatoxin M1 showed some affinity within the range from 1 X 10-7 to 1 X 10-6 molar. Compared with the endogenous estrogen, 17beta-estradiol (100%) and the stilbene derivate dienoestrol (10%) the estrogenic potency of aflatoxin M1 is about 0.5%. The presence of the free alcoholic OH-group on C-atom 4 seems to be essential for the binding of aflatoxin M1 to the receptor. Replacement of the OH group by another H-atom (as in Aflatoxin B1, G1 and G2) eliminated binding affinity.

166. THE EFFECTS OF STEROID HORMONES AND CARCINOGENS ON THE INTERACTION OF MEMBRANES WITH POLYSOMES.

Rabin BR, Blyth CA, Doherty D, Freedman RB, Roobol A, Sunshine G, Williams DJ
J Clin Pathol; 27(7):51-59 1974

Evidence indicates that free and bound polysomes are probably involved in the biosynthesis of different classes of proteins, and that the ratio of free to bound polysomes is a function of the hormonal status of the organism. Steroid hormones (or equivalent materials extracted from polysomes or membranes) are required for the occurrence of membrane-polysome interactions. The requirements are sex-specific and are for estradiol (or equivalent material extracted from female polysomes) in the male and for testosterone (or equivalent material extracted from male polysomes) in the female. Cross-sex experiments suggest that there may also be a requirement for the other hormone. There are specific 'tight sites' (sites at which the membrane is bound very tightly) for estradiol on the male and for testosterone on the female smooth membranes. **These sites are destroyed irreversibly in vivo and in vitro by aflatoxin B1.** The estradiol and testosterone, or equivalent substances, are already present in rough reticulum. Aflatoxin B1 causes the irreversible displacement of polysomes from rough membranes in vivo and in vitro, the evidence being consistent with the blocking of a specific hormone site. The steroid sites on both rough and smooth membrane can be specifically protected from destruction by the carcinogen by the hormone involved. A number of other carcinogenic chemicals produce the same effects as aflatoxin B1. Some of these compounds cause degranulation, the correlation with carcinogenicity being complete. The presence of NADPH is an absolute requirement. The mechanism of carcinogen activation and subsequent degranulation may involve the cytochrome P450-flavoprotein enzyme present in microsomal membranes. The complete inhibition of the overall degranulation by low concentrations of estradiol suggest that it is the steroid-specific site, responsible for maintaining membrane-polysome complex integrity; this is attacked by the activated carcinogen.

167. INTERACTION OF DRUGS WITH MICROTUBULE PROTEINS.

Wilson L, Bamburg JR, Mizel SB, Grisham LM, Creswell KM
Dept. Pharmacol., Stanford Univ. Sch. Med., Stanford, Calif.
Fed Proc; 33(2):158-166 1974

Microtubule function can be impaired by different mechanisms of antimitotic drugs. Published studies on the biochemical mechanism of action of colchicine on microtubule function are described. Tubulin is a "drug receptor" for a number of these drugs; there is one high affinity binding site on tubulin for colchicine, and current evidence suggests the binding of colchicine at this site prevents microtubule polymerization. Studies on the mechanism of action of other "spindle poisons" are also reviewed. The binding site for podophyllotoxin is the same as that for colchicine; the Vinca alkaloids (vinblastine and vincristine) prevent microtubule assembly by binding to tubulin at different sites, forming highly regular microtubule crystals within cells. **Griseofulvin** acts by a different mechanism; it may affect a functional property of assembled microtubules (rather than preventing their assembly) or may act on other processes essential for normal completion of cell division. (34 refs)

168. THE EFFECT OF FUNCTIONAL GROUPS ON THE INTERACTION OF AFLATOXINS B1 AND G1 WITH STARCH, CELLULOSE, AND SEVEN CELLULOSE DERIVATIVES.

Uwaifo AO, Bassir O
Dept. Biochemistry, Univ. Ibadan, Ibadan, Nigeria
Biochem Pharmacol; 26(9):863-866 1977

The interaction of several polysaccharides with aflatoxin B1 and aflatoxin G1 was studied by equilibrium dialysis to determine the effects of different functional groups on binding. Polysaccharides were chosen as macromolecular models because the glucose chain is basically a constant moiety in their structure, and any differences in their interaction would be due to the functional groups. The results showed a marked variation in binding. No binding was observed with cellulose, carboxymethyl cellulose, phosphocellulose, and cellulose acetate. However, the amino derivatives of cellulose - ECTEOLA cellulose, triethylaminoethyl cellulose, and diethylaminoethyl cellulose - bound the aflatoxins. In general, aflatoxin B1 bound more strongly to the polysaccharides than aflatoxin G1. (18 Refs)

C. Modification of Metabolic Processes and Biochemical Pathways Following Exposure to Mycotoxins

169. CARBOHYDRATE METABOLISM IN AFLATOXIN TOXICITY.

Jayanthi Bai N, Ramachandra Pai M, Venkitasubramanian TA
Dept. Biochemistry, T. D. Medical Coll., Alleppey 688 005, India
Indian J Biochem Biophys; 14(1):86-88 1977

The effects of aflatoxins B1, G1, and M1 on carbohydrate metabolism in rat liver and kidney were studied 48 hr after the ip injection of single LD50 doses of the toxins. The aflatoxins failed to elicit any significant changes in hepatic glycogen level, but they increased phosphofructokinase activity in both the liver and kidneys. Pyruvate kinase activity was inhibited by aflatoxin M1 in both tissues. Liver glucose-6-phosphatase activity was reduced by aflatoxin B1 and aflatoxin G1, but the kidney enzyme was not affected. Aflatoxin M1 increased the activity of this enzyme in the kidney, but did not affect it in the liver. Aflatoxins B1 and G1 enhanced the activity of hexose monophosphate dehydrogenase in both tissues, but M1 enhanced it in the kidney but not in the liver. The results indicate that carbohydrate metabolism is affected differently by these toxins. (21 Refs)

170. CARBOHYDRATE METABOLISM IN AFLATOXIN B1 TOXICITY.

Raj HG, Venkitasubramanian TA
Vallabhbhai Patel Chest Inst., Univ. Delhi, India
Environ Physiol Biochem; 4(4):181-187 1974

A study was made of the utilization of glucose by aflatoxin B1-injected one-day-old, male white leghorn chicks. Glycolytic and gluconeogenic enzymes were also studied to assess the extent to which the different processes controlling carbohydrate metabolism are operating in aflatoxin B1-treated chick liver. The birds were injected with aflatoxin B1 (2.7 mg/kg ip) in 0.1 ml of propylene glycol; controls received propylene glycol alone. The birds were sacrificed 48 hr after toxin administration. Glycogenesis in vivo was studied in the livers of control and treated chicks according to the procedure of Langley. Standard methods were used for the glucose tolerance tests and for the assay of enzymes. Aflatoxin B1 resulted in a marked inhibition of hepatic glycogenesis in vivo, and an increased fasting blood sugar level. Blood sugar levels reached a peak of 90 min after glucose administration in both groups; however, in the treated chicks the blood sugar levels 45 min after glucose administration and at subsequent periods were markedly elevated as compared with controls. These differences are suggestive of lowered glucose tolerance in aflatoxin B1-treated chicks. Aflatoxin markedly inhibited the activities of some glycolytic enzymes (hexokinase, phosphoglucoisomerase, fructose-diphosphate aldolase, and pyruvate kinase). The activities of the key enzymes of gluconeogenesis (pyruvate carboxylase and phosphoenol-pyruvate carboxykinase) were significantly enhanced by aflatoxin, while the activity of fructose diphosphatase was unaffected. The results suggest that prolonged aflatoxin poisoning may lead to decreased food consumption, increased fat oxidation, and coma as a result of severe deprivation of carbohydrate utilization.

171. DISTURBANCES IN TRYPTOPHAN METABOLISM AFTER A SINGLE DOSE OF AFLATOXIN B1 AND CHRONIC INTOXICATION WITH CARBON TETRACHLORIDE.

Lemonnier FJ, Scotto JM, Thuong-Trieu C
Groupe U56, INSERM, Hospital Bicetre, France
J Natl Cancer Inst; 53(3):745-749 1974

Female Wistar rats were given a single dose (half the LD50) of aflatoxin B1, by gastric gavage, either alone or in combination with chronic intoxication with carbon tetrachloride (CCl4, daily inhalation for 8 months). Histologic and biochemical studies were conducted during the subsequent 30 months. An L-tryptophan load (10 mg/100 g rat, by gavage) was administered and the urinary excretion of certain of its metabolites was evaluated. The activity of hepatic tryptophan-oxygenase and the quantity of nucleic acids were determined when the animals were killed. The percentage of animals with abnormal test results was significantly higher in the groups given aflatoxin or CCl4+aflatoxin than in a control

group. Furthermore, the association of the two toxic substances increased all biochemical parameters and induced earlier and more intense histologic modifications. The number of hepatomas, however, was virtually identical in all groups. The biochemical anomalies observed probably resulted more from the administration of the toxic substance(s) than from the neoplastic process.

172. EFFECT OF HEPATOCARCINOGENS ON THE BINDING OF GLUCOCORTICOID-RECEPTOR COMPLEX IN RAT LIVER NUCLEI.

Kensler TW, Busby WF, Davidson NE, Wogan GN
McArdle Laboratory for Cancer Research, University of Wisconsin Medical Center, Madison, Wis. 53706.
Cancer Res; 36(12):4647-4651 1976

The effects of a number of carcinogens and hepatotoxins on the binding kinetics of the interactions of glucocorticoid-cytosol receptor complex with nuclear acceptor sites in rat liver were investigated. Both the apparent concentration of nuclear binding sites and the K subd were significantly diminished following treatment of rats with sublethal doses of the carcinogens aflatoxin B1, diethylnitrosamine, dimethylnitrosamine, thioacetamide, 3'-methyl-4-dimethylaminoazobenzene, 4-dimethylaminoazobenzene and 3-methylcholanthrene. Treatment with actinomycin D resulted in a slight reduction in the apparent concentration of nuclear acceptor sites but had no effect on the nuclear binding K subd. The hepatotoxic but noncarcinogenic analgesic, acetaminophen, as well as the weakly toxic aflatoxin B1 cognate, aflatoxin B2, were without effect on the kinetics or binding capacity of glucocorticoid-nuclear acceptor site interaction. These experiments suggest that chemically induced alteration of functional glucocorticoid binding sites on chromatin may be involved in the biochemical effects produced in liver by carcinogens of several chemical types. This experimental model may provide a useful approach for further elucidation of early events in carcinogenesis. (Author Abstract)

173. AFLATOXIN EFFECTS ON GLUCOCORTICOID NUCLEAR BINDING IN RAT LIVER.

Kensler TW, Davidson NE, Busby WF, Wogan GN
Massachusetts Institute of Technology, Cambridge, Mass. 02139
Proc Am Assoc Cancer Res; 16:84 1975

Aflatoxins block the glucocorticoid induction by de novo synthesis of the rat liver enzymes tyrosine aminotransferase (TAT) and tryptophan pyrrolase (TP). Glucocorticoid binding to cytosol receptors is an essential component of the induction process (Beato et al, BBRC 47: 1464, 1972). This induction phenomenon is probably mediated also by interaction of glucocorticoid-receptor complexes with nuclear acceptor sites. Using glucocorticoid binding as a probe of chromatin function, the ability of (^{3}H)-dexamethasone-cytosol receptor complexes to bind to rat liver nuclei prepared from 100 gm adrenalectomized male Fischer rats pretreated with saline, DMSO or aflatoxin B1 (AFB1) (1mg/kg,ip, 2 hrs prior to sacrifice) was investigated. The apparent concentration of nuclear binding sites (rbo moles (^{3}H)-dex bound/mg DNA), measured by incubating at 0 degrees C (^{3}H)-dexamethasone-cytosol receptor complexes with nuclei isolated from the same rats, diminished 30% in AFB1 treated rats compared to both saline and DMSO controls. Kd values for the binding reaction are also reduced by 30% following AFB1 treatment. Heterologous or 'cross-over' experiments suggest that aflatoxin exerts its effect primarily at the nuclear level rather than on dexamethasone-cytosol receptor formation. Nuclear binding capacity of aflatoxin-cytosol macromolecule complexes is also being investigated. (Author Abstract)

174. AFLATOXIN INHIBITION OF GLUCOCORTICOID BINDING CAPACITY OF RAT LIVER NUCLEI.

Kensler TW, Busby WF, Davidson NE, Wogan GN
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass 02139 (USA)
Biochim Biophys Acta; 437(1):200-210 1976

The effect of aflatoxin B1 on the binding capacity of rat liver cytoplasmic glucocorticoid receptors and the nuclear binding of the activated receptor complex was investigated. No alterations in the kinetics of (^{3}H) dexamethasone-cytosol receptor complex formation were noted 2 hr after treatment with 1 mg/kg aflatoxin B1. However, a 33% decrease in the concentration of nuclear acceptor sites and a 24% decrease in the glucocorticoid receptor-nuclear binding equilibrium constant of dissociation was observed. This response was near maximal at 2 hr and persisted for at least 36 hrs. Inhibition of nuclear binding capacity was directly related to aflatoxin B1 dose with a correlation coefficient of 0.99. Actinomycin D treatment (0.1 mg/kg) resulted in a slight reduction (16%) in the concentration of nuclear acceptor sites but had no effect on the nuclear binding dissociation constant. Administration of (^{3}H) dexamethasone to aflatoxin B1-treated rats produced a similar pattern of glucocorticoid binding distribution in vivo to that observed in vitro. No differences in (^{3}H) dexamethasone-cytoplasmic receptor binding between control and aflatoxin B1-treated rats were found, whereas nuclear (^{3}H) dexamethasone binding was reduced 34% by aflatoxin B1 treatment. (Author Abstract)

175. AFLATOXIN EFFECTS WITH LIQUID CRYSTALLINE CHOLESTERYL ESTERS OF DUCKLING SERA.

Lynd JQ, Lynd FT
Soil Microbiology, Department of Agronomy, Oklahoma State University, Stillwater, Oklahoma 74074
Tex Rep Biol Med; 33(3):423-432 1975

An objective of this study was to determine indicator parameters of blood sera that are symptomatic for degenerative hepatic cholesterogenesis and lipid transformations induced with carcinogenic mycotoxins. A technique was developed for electrophoretic separation of liquid crystalline cholesteryl esters within beta lipoprotein fractions of duckling sera. These birefringent moieties decreased to 110 ug/mg low-density lipoprotein during 10-day bioassay periods for normal, nontoxin fed ducklings, but remained unchanged-179-189 ug/mg-for toxin-fed principals ingesting 0.988 ug aflatoxin/g feed throughout the bioassays. Their composition was principally cholesteryl esters with liquid crystalline characteristics determined by the oleate and linoleate content. Increased cholesterol levels-11.4% to 17.1% within sera of the nontoxin group-resulted in more frequent spherulitic mesoforms. The 0.9% to 1.8% taurochenodesoxycholate content within all sera samples induced stable cubic liquid crystalline forms. With polarized microscopy, cholesteric anisotrophs of sera were unique in composition and iridescent visage for each aflatoxin level regime. This specific dicroism was correlated with the corresponding hepatic histology. (Author Abstract)

176. EFFECT OF AFLATOXINS ON OXIDATIVE PHOSPHORYLATION BY RAT LIVER MITOCHONDRIA.

Ramachandra-Pai M, Jayanthi-Bai N, Venkitasubramanian TA
Vallabhbhai Patel Chest Inst., Univ. Delhi, India).
Chem Biol Interact; 10(2):123-131 1975

The in vitro effects of aflatoxins M1, B1, and G1 on oxidation and phosphorylation by rat liver mitochondria were studied. Liver mitochondria isolated from nonfasted albino rats were incubated with the aflatoxins dissolved in DMSO, using succinate as substrate. Respiration and phosphorylation were measured polarographically at 30 C. All of the aflatoxins inhibited electron transport at concentrations of 10-4 M. The site of inhibition was between cytochrome b and cytochrome c or c1. In addition, aflatoxin M1 uncoupled oxidative phosphorylation at 10-6 M and reduced the ADP:O ratio, while aflatoxin B1 (10-6 M) uncoupled oxidative phosphorylation, but had no effect on the ADP:O ratio. The ADP:O ratio was decreased by aflatoxin B1 (10-5 M). Aflatoxin G1 was able to uncouple oxidative phosphorylation only at relatively high concentrations (10-4 M). Preincubation of rat liver mitochondria with these toxins resulted in respiratory inhibition and uncoupling. It is likely that the aflatoxins exert their uncoupling effect via binding at specific sites responsible for oxidative phosphorylation, although they may instead effect a gross change in the structure of the mitochondrial membrane.

177. COMPARATIVE STUDIES ON LYSOSOMAL ACTIVITIES IN MYCOTOXIN-TREATED BIRD EMBRYOS.

Adekunle AA, Elegbe RA
Dept. Biochem. Physoil., Univ. Ibadan, Nigeria
Environ Physiol Biochem; 4(6):289-293 1974

The effect of aflatoxin B1 on the livers of five species of bird embryos was investigated in order to establish that lysosomal integrity of aflatoxin-treated animals is species-dependent. Aflatoxin B1 in doses of 0 to 0.6 microg/egg in 0.05 ml propylene glycol was introduced into the yolk sac of the embryos of domestic fowl (Gallus domesticus) of White Rock breed; duck (Cairina moschata, Muscovy); guinea fowl (Numida meleagris, Kano): domestic pigeon (Columbia livia, Locale) and turkey (Meleagris gallipavo, Sokoto) after nine days of incubation. Homogenates of the liver were made from birds hatching from 2-2.5 weeks after treatment. The acid phosphatase activity was determined by measuring the amount of inorganic P set free in the presence of 0.05 M beta-glycerophosphate and 0.05 M acetate buffer adjusted to pH 5.0 with HC1. Lysosomes of animals treated with aflatoxin B1 in vivo showed evidence of leakage as judged by specific activities of two marker enzymes, acid phosphatase and beta-glucuronidase. The modes of leakage varied from one species to another. Specific activities were significant in domestic fowl and guinea fowl, but not in duck, domestic pigeon and turkey. Embryonic tissue damage caused by aflatoxin B1 interaction may lead to the observed high mortality in these three species. In the same family of different species the percentage increase in lysosomal activity induced in intoxicated livers may vary from one species to the other. The results support the idea that hepatocellular changes produced by aflatoxin in birds are mediated through primary damage to lysosomes.

178. THE EFFECT OF AFLATOXIN B1 ON THE ENZYMATIC ACTIVITY OF BACILLUS THURINGIENSIS (BERLINER).

Boutibonnes P
Laboratoire de Physiologie Bacterienne II, Universite de
Caen, Caen, Cedex 14032, France
Can J Microbiol; 23(7):931-932 1977

At 20 C, aflatoxin B1, at a sublethal dose, decreases the activity of alkaline phosphatase (EC 3.1.3.1), alpha-glucosidase (EC 3.2.1.20), esterase (EC 3.1.1.1), chymotrypsin (EC 3.4.21.1), leucine aminopeptidase (EC 3.4.11.1), and phosphoamidase (EC 3.9.1.1) biosynthesis in Bacillus thuringiensis . In contrast, at 41 C no significant decrease was observed. At this temperature,the mycotoxin is not destroyed or metabolized and bacterial cells are resistant to the toxin. (Author abstract) (11 Refs)

179. EFFECT OF AFLATOXIN B1 ON HEPATIC POLYRIBOSOMES AND PROTEIN SYNTHESIS IN THE RAT.

Sidransky H, Verney E, Murty CN, Sarma DS, Reid M
Dept. Pathology, George Washington Univ. Medical
Center, 2300 Eye St., N.W., Washington, DC 20037
Chem Biol Interact; 18(1):69-82 1977

The effect of aflatoxin B1 (AFB: 6.0 mg/kg ip) on hepatic polyribosomes (free and membrane-bound), protein synthesis, and activity of initiation factors in rats within 3, 6, and 12 hr after treatment was investigated. AFB led to a progressive disaggregation of free and membrane-bound polyribosomes, inhibition of in vitro protein synthesis by both populations of polyribosomes, and to defective ribosomes, particularly the 60S ribosomal subunits, of both types of polyribosomes. AFB had little or no effect on the activities of initiation factors. In comparative studies with actinomycin D (A-D), AFB decreased RNA synthesis by 73%, decreased in vitro protein synthesis by 58%, and prevented the induction of hepatic tryptophan pyrrolase by cortisone, but A-D decreased RNA synthesis by 80%, in vitro protein synthesis by 54%, and prevented the induction of tryptophan pyrrolase. However, there was progressive disaggregation of only the free polyribosomes afer A-D. (40 Refs)

180. INHIBITION OF NUCLEOSIDE TRANSPORT BY AFLATOXINS AND STERIGMATOCYSTIN.

Kunimoto T, Kurimoto Y, Aibara K, Miyaki K
Dept. Biomed. Res. Food, Natl. Inst. Health, Tokyo
Cancer Res; 34(5):968-973 1974

Aflatoxin B(1), B(2), G(1), and G(2) and sterigmatocystin were added to cultures of L5178Y and HeLa cells (5-7.5 x 10^5 cells/ml) incubated with ^{3}H-uridine (0.6 microCi/ml) or ^{3}H-thymidine (0.5 microCi/ml) at 37 degreesC. The incorporation of ^{3}H-uridine and ^{3}H-thymidine into the acid-soluble fraction of L5178Y cells was inhibited immediately after the addition of aflatoxin B(1) or sterigmatocystin. Incorporation of ^{32}PO(4)**-3 into the acid soluble fraction was not inhibited by these mycotoxins under similar conditions. Aflatoxin B(1) and sterigmatocystin inhibited the uptake of ^{3}H-uridine by HeLa and L5178Y cells at 20 degreesC. A correlation was found between the uptake of ^{3}H-uridine into whole cells and its incorporation into acid-soluble fractions at various conc. (1-30 microg/ml). The inhibition of uridine and thymidine transport was linear and competitive. Mycotoxins had little or no effect on RNA synthesis; HeLa RNA synthesis was only slightly effected by aflatoxin B(1) compared to the inhibition of thymidine uptake, however, sterigmatocystin had no effect on DNA synthesis. (40 refs)

181. INHIBITORY EFFECTS OF MYCOTOXINS ON NA**+-DEPENDENT TRANSPORT OF GLYCINE IN RABBIT RETICULOCYTES.

Ueno Y, Matsumoto H, Ishii K, Kukita K
Laboratory of Microbial Chemistry, Faculty of
Pharmaceutical Sciences, Tokyo University of Science,
Ichigaya, Tokyo 162, Japan
Biochem Pharmacol; 25(18):2091-2095 1976

An inhibitory effect of toxic fungai metabolites on Na**+-dependent transport of (^{14}C)glycine by rabbit reticulocytes was examined. Among twenty-three mycotoxins tested, patulin, a carcinogenic lactone of Penicillium and Aspergillus, caused the most marked inhibition. From comparative experiments with several metabolic inhibitors and SH-blockaders, patulin was presumed to inactivate SH groups of the receptor site for the glycine transport system in reticulocyte membranes. (Author Abstract)

182. HEPATIC ENZYMES IN AFLATOXIN-INDUCED PRECANCER.

Pokrovskii AA, Nikolaieva MI, Lashneva NV, Kravchenko LV, Korovnikov KA, Tutelian VA
Inst. Nutr. Moscow, USSR
Vopr Onkol; 20(9):75-79 1974

Precancerous changes in the rat liver enzymes induced by aflatoxin administration were studied in Wistar male rats injected ip with 40 microg/day of aflatoxin for 30 days (the total dose was equal to that inducing hepatocarcinomas in rats). The animals were sacrificed 9 months after injections were completed. The activity of all lysosomal enzymes decreased significantly, particularly acid RNA-ase, beta-glucosidase and beta-galactosidase (by 37.3%, 32.3%, and 48.4%, resp). At the same time a significant increase occurred in the total activity of glucose-6-phosphatase and acetylesterase isoenzymes (29.0 and 13.7%, resp). In addition, a considerable decrease occurred in the rate of NADP-H-dependent lipid peroxidation and in the isoenzymes of NAD-dependent lactate dehydrogenase and phosphofructokinase.

183. MICROSOMAL ENZYME MODIFICATION: ITS RELATIONSHIP TO THE METABOLIC ACTIVATION OF CHEMICAL CARCINOGENS (MEETING ABSTRACT).

Roberfroid MB, Cumps J, Razzouk C
Laboratory for Cellular Biotoxicology, CMTB, University of Louvain, 7369 B 1200 Bruxelles Belgium
Third International Symposium On Detection And Prevention Of Cancer; 70-71 1976

Aryl hydrocarbon hydroxylase (AHH) and AHH induction have been proposed as key elements in hydrocarbon-induced carcinogenesis. Some correlation has been suggested between enzyme inducing potency and carcinogenic activity of polycyclic hydrocarbons. It has thus been hypothesized that AHH induction is probably the first step in chemical carcinogenesis. Injecting aflatoxin B1, 2 and 4 acetylaminofluorene we have analyzed both their biochemical effects on the liver and their specific effects on the cytochrome P450 dependent microsomal enzymic activities. For studying the biochemical effects we have applied De Duve's procedure for tissue fractionation and analyzed the subcellular fractions measuring the pattern of distribution, the latency and the activity of various marker enzymes. The specific effects on the microsomes have been characterized by kinetic analysis of benzpyrene hydroxylase and 2-acetylaminofluorene N-hydroxylase applying respectively the Dehnen's fluorometric procedure and an original gas chromatographic assay we developed for the N-hydroxylated derivatives. Our results show that short term treatments with chemical carcinogens do not significantly modify the biochemical properties of the liver cells as measured by the integrity of the subcellular organelles. The same treatments however are able to statistically modify the properties of the mixed function oxydase enzyme system. Those modifications imply changes in the K subm for the carcinogens. Their relationships to the chemical carcinogenesis will be discussed. (Author Abstract)

184. HISTOCHEMICAL DEMONSTRATION OF INCREASED ACTIVITY OF GAMMA-GLUTAMYL TRANSPEPTIDASE IN RAT LIVER DURING HEPATOCARCINOGENESIS.

Harada M, Okabe K, Shibata K, Masuda H, Miyata K, Enomoto M
Dept. Pathology, St. Marianna Univ. Sch. Medicine, Kawasaki 213, Japan
Acta Histochem Cytochem; 9(2):168-179 1976

Gamma-glutamyl transpeptidase (g-GTP) activity was studied during hepatocarcinogenesis induced by aflatoxin B1 and the N-hydroxy derivative of N-2-fluorenylacetamide (N-OH-FAA) in male Sprague-Dawley rats. Control rats had g-GTP activity in bile duct cells and occasionally along the bile canaliculi; there was no activity in the hepatocytes. Proliferating bile duct cells, including oval cells and cholangiocarcinoma cells, hepatocytes of hyperplastic areas and nodules, and hepatocellular carcinoma induced by N-OH-FAA, with or without aflatoxin B1, all had g-GTP activity. Hepatocytes of rats treated with aflatoxin B1 alone had mild hyperplastic changes with only focal enzyme activity. The elevation of g-GTP activity in the serum and liver tissue was associated with the appearance of its fetal isoenzyme; this was correlated with the histochemical appearance of g-GTP activity in the liver tissue from the early phase of hepatobiliary carcinogenesis. These results demonstrate the carcino-embryonic nature of g-GTP in the developing cholangiocellular and hepatocellular carcinomas. (28 Refs)

185. EFFECTS OF SUBACUTE AFLATOXIN B1 TREATMENT ON HAMSTER LIVER MICROSOMAL MIXED FUNCTION OXIDASE ACTIVITY. (MEETING ABSTRACT).

Tucker AN, Tang T, Friedman MA, Egle JL
Dept of Pharmacol, Med Col of Va, Va Comm Univ, Richmond, Va 23298
Fed Proc; 36(3):940 1977

Hamsters were treated daily for 3 or 7 days with aflatoxin B1 (1 mg/kg, ip). The 9,000 X g supernatant (S-9) was used for mutagenicity studies in the Ames test and for measurement of aminopyrine demethylase and aniline hydroxylase activities. Microsomes (washed, 96,000 X g pellet) were isolated from the S-9 fractions and used in determination of type I (aminopyrine) and type II (aniline) binding spectra. Aflatoxin B1 caused a 52% decrease in microsomal protein content at both times. Aminopyrine demethylase and aniline hydroxylase activities decreased and could be largely accounted for by the decreased yield of microsomal protein. The specific activity of cytochrome P-450 in the microsomes was only slightly depressed. Microsomes from aflatoxin B1 treated hamsters failed to show characteristic type I binding spectra, although type II spectra were similar to controls. The magnitude of the response studied did not increase with time of treatment. Feeding hamsters a 1% piperonyl butoxide diet failed to alter the microsomal response to the aflatoxin B1 injections. (Author Abstract)

186. ENHANCEMENT OF THE ACTIVITY OF BEEF MUSCLE LACTATE DEHYDROGENASE BY AFLATOXIN B1 IN VITRO.

Lu FJ, Chiou HY, Hsu CY, Ling KH
Coll. Med., Natl. Taiwan Univ., Taipei
J Formosan Med Assoc; 73(1):16-21 1974

The effect of aflatoxin B1 on the activity of beef muscle lactate dehydrogenase (LDH) was studied in vitro. LDH was incubated with aflatoxin B1 purified from Aspergillus flavus. In a complete system consisting of pyruvate, LDH, and NADH, the activity of LDH was activated by the addition of aflatoxin B1; the degree of enhancement was dependent on the molar ratio of enzyme to aflatoxin, maximum enhancement being obtained at a ratio of 4:1. When no substrate (pyruvate) was present in the system, there was no enzymic activity either in the presence or absence of aflatoxin. As shown by elution on a Sephadex G-100 column, aflatoxin B1 was not firmly bound to LDH after one hr of incubation. The absorption spectrum of LDH-aflatoxin B1 was changed by the presence of pyruvate in the incubation medium. The results suggest that the mechanism of interaction between aflatoxin B1 and LDH is different in vitro and in vivo.

187. INCORPORATION OF 2**-14C ACETATE INTO LIPIDS OF MINK (MUSTELA VISION) LIVER AND INTESTINE DURING IN VITRO AND IN VIVO TREATMENT WITH AFLATOXIN B1.

Chou CC, Marth EH
Dept. Food Science, Food Res. Inst., Univ. Wisconsin-Madison, Madison, WI 53706
Appl Microbiol; 30(6):946-950 1975

The effects of aflatoxin B1 on in vitro and in vivo incorporation of 2C14 acetate into lipids of mink (Mustela vision) liver and intestines was studied. In vitro, a dose of aflatoxin B1 as small as 7.5 microg/ml caused a 20% reduction in incorporation of 2C14 acetate into total lipids compared with controls; 180 microg of aflatoxin B1 caused a 76% decrease in the synthesis of lipids from the radioactive precursor. A similar inhibition of lipid synthesis by aflatoxin was also observed with tissues from mink intestines and fatty liver. The degree of inhibition (19-84% for tissues from intestines and 19-64% for tissue from fatty liver) depended on the amount of aflatoxin B (7.5-180 microg) present in the medium. Inhibition was generally nonspecific for the different lipid classes (triglyceride, free fatty acid, cholesterol, and phospholipids), although a higher reduction in incorporation of C14 in cholesterol (67-91% inhibition at various aflatoxin doses) was observed. In vivo, a substantially increased amount of C14-labeled lipids was found in the livers of mink injected with 600 microg aflatoxin B1/kg body wt 20, 28, and 40 hr earlier. There was appreciable difference in incorporation of C14 acetate in liver lipids between the toxin-treated and control animals at 4 and 10 hr after aflatoxin B1 injection, suggesting that after entering the body, aflatoxin B1 or its metabolites required some time to influence the system and cause changes in lipid metabolism. The increase in C14-labeled lipids after aflatoxin B1 administration appeared in all lipid classes examined. The differences in the dosages of aflatoxin may have contributed to the different results in vitro and in vivo. The smallest dose, 7.5 microg/ml medium, used for in vitro studies is much greater than the dose (600 microg/kg) administered to mink for the in vivo study. Two possible causes for the increased amounts of C14-labeled lipids observed in the liver in in vivo experiments are suggested: (1) aflatoxin B1 or its derivatives alters the metabolic rate in the liver to cause reduced oxidation or increased lipid synthesis; and (2) aflatoxin B1 or its metabolites caused a change in the mobility of lipids. (14 refs)

D. Effects of Mycotoxins on Synthesis of RNA and Proteins

188. ACUTE EFFECTS OF ETHIONINE, DIMETHYLNITROSAMINE OR AFLATOXIN B1 ON THE ACTIVITIES OF INITIATION FACTORS AND RIBOSOMES IN RAT HEPATIC PROTEIN SYNTHESIS.

Murty CN, Verney E, Sidransky H
University of South Florida, Tampa, FL 33620
Proc Am Assoc Cancer Res; 17:73 1976

The present investigation studied the activities of initiation factors and ribosomes of livers of rats treated acutely with ethionine, dimethylnitrosamine (DMN) or aflatoxin B1 using an in vitro assay in which poly(U)-directed polyphenylalanine synthesis was measured at low Mg^{2+} concentration. In rats treated with ethionine (1 mg/g B.W.; 4 or 6 hr) or DMN (0.1 mg/g B.W.; 4, 8 or 15 hr) the activities of both initiation factors and ribosomes were decreased in polyphenylalanine synthesis compared to controls. However, treatment with aflatoxin B1 (0.06 mg/g B.W.; 6 or 12 hr) revealed decreased activities of ribosomes but not of initiation factors in polypeptide synthesis. Administration of ethionine, DMN or aflatoxin B1 in these experiments caused disaggregation of hepatic polyribosomes. Administration of adenine sulfate (0.3 mg/g B.W.) plus methionine (0.9 mg/g B.W.) 4 hr after ethionine treatment and 2 hr before killing and administration of diethyldithiocarbomate (0.35 mg/g B.W.) 3/4 hr before DMN treatment and 4 3/4 hr before killing reversed or prevented the impairment of the activities of initiation factors and of ribosomes as well as the disaggregation of hepatic polyribosomes by each of the two carcinogenic compounds. (Author Abstract)

189. METABOLIC CHANGES CAUSED BY AFLATOXIN B1 AND EFFECTS ON NUCLEIC ACID AND PROTEIN SYNTHESIS.

Moule Y
Inst. Sci. Res. Cancer, Villejuif, France
Ann Nutr Aliment; 28(4):375-384 1974

Deviations, triggered by a single dose of aflatoxin B1 (1 mg/kg), in RNA, DNA and protein synthesis of rat liver were studied to demonstrate how a specific fundamental biochemical procedure can uncover toxicity of a substance and elucidate its mechanism of action. The inhibition of RNA synthesis seen upon administration of C14-orotic acid, and the fact that RNA polymerase of isolated rat liver nuclei is strongly inhibited when treated in vivo with aflatoxin demonstrate that the transcription systems, located in the nucleolus and in the nucleoplasm, have been affected by the toxin. Aflatoxin also inhibits synthesis of liver proteins. A biphasic effect and intense early inhibition are observed. The inhibition of protein synthesis is due to the alteration of RNA synthesis by the mycotoxin. The early effect of aflatoxin is interpreted as a manifestation of toxicity and led to the hypothesis that aflatoxin acts directly on the polysomes. Inhibition of DNA synthesis was proven by inhibition of replication.

190. COMPARATIVE IN VITRO BIOCHEMICAL EFFECTS OF PATULIN AND AFLATOXIN B1. (MEETING ABSTRACT).

Peters EL, Keys JE, Friedman L
Div of Toxicology, FDA, Washington, D C 20204
Fed Proc; 36(3):397 1977

The mycotoxins patulin (P) and aflatoxin B1 (A) are known to affect cellular respiratory activity and macromolecular metabolism. Liver slices from male rats, hamsters, mice, rabbits, chickens and Rhesus monkeys were exposed to A and P in Krebsglucose medium for 1 hr at 37 degrees C; trout liver slices were exposed at 18 degrees C. Incorporation of ^{14}C-orotic acid and ^{3}H-leucine into RNA and protein, respectively, and ^{14}CO2 production from ^{14}C-glucose were measured. In all cases, RNA and protein syntheses were inhibited by P (12.5-50 mug/ml) to a greater degree than was glucose oxidation. In a comparison of livers from rats, hamsters, mice, rabbits and chickens, rat liver was generally most sensitive to P and rabbit liver was least sensitive. P (9 and 18 mg/kg per os) inhibited in vivo hepatic protein synthesis (13 and 49%) and produced disaggregation of liver polysomes in rats. In vitro RNA synthesis was about equally inhibited by A (0.125-1.0 mug/ml) in livers of trout and rats, both A-sensitive species; mouse livers were relatively resistant. **Protein synthesis**, however, was more severely and consistently inhibited by A in trout liver; trout liver glutathione was also about half that of mouse or rat liver. A-induced inhibition of RNA synthesis varied widely in individual monkey livers. Sensitivity to in vitro macromolecular synthesis inhibition by A and P is species-dependent, and, at least for A, is related to in vivo susceptibility. (Author Abstract)

191. MECHANISM OF ACTION OF THE 12,13-EPOXYTRICHOTHECENE, ANGUIDINE, AN INHIBITOR OF PROTEIN SYNTHESIS.

Liao LL, Grollman AP, Horwitz SB
Department of Pharmacology and Cell Biology, Albert Einstein College of Medicine, Bronx, N.Y. 10461 (U.S.A.)
Biochim Biophys Acta; 454(2):273-284 1976

Anguidine, muconomycin A, T-2 toxin, crotocin and trichodermin, a group of 12,13-epoxytrichothecenes, inhibit protein synthesis in HeLa cells and in rabbit reticulocyte lysates. These five mycotoxins can be divided into two groups on the basis of the reversibility of their effects in HeLa cells, and kinetics of inhibition and effects on polyribosome structure in rabbit reticulocyte lysates. Anguidine, muconomycin A and T-2 toxin are irreversible inhibitors of protein synthesis; crotocin and trichodermin are reversible inhibitors of protein synthesis. After addition of low concentrations (1 uM) of anguidine, muconomycin A or T-2 toxin to rabbit reticulocyte lysates, polyribosomes are broken down to monosomes. At higher concentrations, 1 mM, these drugs begin to freeze the polyribosomes. Crotocin and trichodermin freeze the polyribosomes at a concentration of 10 uM. We conclude that anguidine, muconomycin A and T-2 toxin act primarily as inhibitors of initiation of protein synthesis, whereas crotocin and trichodermin inhibit the process of chain elongation. (Author Abstract)

192. TRANSFER RNA METHYLASE ACTIVITY AND CAPACITY DURING AFLATOXIN B1-INDUCED HEPATOCELLULAR CARCINOGENESIS.

Busby WF, Paglialunga S, Newberne PM, Wogan GN
77 Massachusetts Avenue, Room 56-212, Massachusetts Institute of Technology, Cambridge, Mass. 02139
Cancer Res; 36(6):2013-2018 1976

Transfer RNA methylase (tRNA methylase) activity and capacity were monitored in whole-rat-liver preparations during the induction of hepatocellular carcinomas by an 8-week aflatoxin B1 dosing regimen that produced minimal toxic effects. Significant phases of elevated tRNA methylase capacity occurred at 6 to 9 weeks (20%) and 24 to 29 weeks (40%). No significant change in tRNA methylase activity was noted over the course of the 55-week experiment. Higher aflatoxin B1 doses, producing acute toxic liver damage, resulted in elevated tRNA methylase activity (50%) and capacity (30%) at least as early as 1 week after dosing. Experiments with individual nodular lesions excised from livers of rats continuously fed a diet containing 2 ppm aflatoxin B1 demonstrated similarly elevated tRNA methylase activities and capacities in hyperplastic (preneoplastic) nodules, with and without histological evidence of carcinoma. (Author Abstract)

193. A DIPLOID RAT LIVER CELL CULTURE. 2. EFFECT OF AFLATOXIN B1 ON RIBONUCLEIC ACID SYNTHESIS.

Bausher J, Schaeffer WI
Univ. Vermont, Coll. Med., Burlington
In Vitro; 9(4):294-301 1974

The effect of aflatoxin B1 on rRNA and total cellular RNA synthesis was studied by sucrose gradient analysis in two rat liver cell cultures. One culture was a diploid line, RL-RP-C, and the other was an established heteroploid line, R-L-T. The RNA synthesis was monitored by using a simultaneous 5-3Huridine and Lmethyl-14C methionine label. In both cell lines, rRNA was the type of RNA most affected by treatment with 50 microg per ml of aflatoxin B1. Synthesis of rRNA in the RL-PR-C line was inhibited by 14%, but the R-L-T line was inhibited by 75% after a two hr incubation with aflatoxin. In the R-L-T culture, the rate of synthesis of rapidly labeled nonmethylated RNA was reduced by 58% while in the diploid cell line the rate of synthesis was stimulated by 75%. This study showed two effects of aflatoxin B1 on RNA synthesis in rat liver cells grown in vitro; one effect was the preferential inhibition of rRNA synthesis, and the other effect was an increase in the rapidly labeled nonmethylated RNA found in the cells of the RL-PR-C cells.

194. MECHANISM OF AFLATOXIN B1 INHIBITION OF RAT LIVER NUCLEAR RNA SYNTHESIS (MEETING ABSTRACT).

Yu FL, Fang J, Wisniewski D
Thomas Jefferson Univ, Philadelphia, Pa. 19107
Proc Am Assoc Cancer Res; 18:88 1977

The mechanism of aflatoxin B1 induced inhibition of rat liver nuclear RNA synthesis in vivo was investigated. Two hrs. after aflatoxin B1 injection (0.3 mg/100 g.b.w.), rat liver nuclear and nucleolar RNA synthesis were inhibited 70% and 90% respectively. In order to establish whether this inhibition was the result of impairment of DNA template function or of inhibition of RNA polymerase activity per se, or both, the free and engaged RNA polymerases were separately assayed by three different methods under conditions that were free from the influence of their endogenous templates. The results show that although the total activities of RNA polymerase I and III were not inhibited, aflatoxin B1 did bring an increase in the free form and a decrease in the engaged form of these enzymes. The activities of both the free and engaged forms of RNA polymerase II were decreased. Since RNA polymerase I, II and III are believed to be responsible for ribosomal, messenger, and transfer RNA synthesis respectively, these results are interpreted to mean that aflatoxin B1 induced inhibition of ribosomal and transfer RNA synthesis is a result of the impairment of the DNA template function, while the inhibition of messenger RNA synthesis is believed, mainly, a result of the selective inhibition of the enzyme activity of RNA polymerase II. (Author Abstract)

195. SELECTIVE INHIBITION OF RAT HEPATIC NUCLEAR RNA POLYMERASE II BY ACTINOMYCIN D IN VIVO (MEETING ABSTRACT).

Yu FL
Dept of Biochem Thomas Jefferson Univ, Philadelphia, Pa 19107
Fed Proc; 36(3):807 1977

After reporting the existence of free and engaged RNA polymerases in rat hepatic nuclei and nucleoli (Nature, 251, 344, 1974), I proposed the 'RNA polymerase cycle' (BBA, 395, 329, 1975). Actin D was used as a probe to test the validity of this model. It was reasoned that when Actin D blocks the initiation and causes premature termination of RNA polymerases from their DNA template sites, the free enzyme pool should be increased and the engaged enzyme level decreased. 30 min after Actin D injection (250 mug/100 gbw), rat hepatic nuclear RNA polymerases I and III followed these predicted patterns. Both the free and engaged RNA polymerase II were inhibited. In vitro addition of Actin D also inhibited RNA polymerase activity when measured with Actin D non-reacting templates, poly(dA-dT) or poly(dI-dC); but the concentrations required were too high to account for the observed effect, in vivo. Also, the inhibition was non-selective. Since RNA polymerase II is believed to be metabolically stable, and the DNA binding carcinogens aflatoxin B1 and N-OH-2-AAF have both produced similar effects, I propose, as a general mechanism in response to the impairment of DNA template function, that an inhibitor is released which selectively inhibits the transcriptional activity of RNA polymerse II. (Author Abstract)

196. FORMATION OF ACTIVE 40 S RIBOSOMAL SUBUNITS IN LIVER IN THE PRESENCE OF AFLATOXIN B1.

Moule Y, Sarasin A
Inst. Sci. Res. Cancer, Villejuif, France
Chem Biol Interact; 9(1):1-6 1974

The formation of 40S ribosomal particles in the presence of aflatoxin B1 and their incorporation in vivo into polysomes was studied using liver homogenates from male Wistar rats (Commentry strain). The rats were given ip injections of 14C-orotic acid and, 3 min later, aflatoxin B1 (1 mg/kg). They were killed 7 hr later. Total RNP (polysomes + monosomes + ribosomal subunits) were obtained from the liver homogenates of control and aflatoxin-treated animals. The polysomes were dissociated by puromycin incubation, and the buoyant density of labeled polysomal 40S subunits was determined. The action of aflatoxin on 45S preribosomal RNA processing resulted in a lack of formation of 60S subunits, while newly synthesized 40S particles continued to be transferred into the cytoplasm as free particles containing labeled 18S RNA, which had an identical sedimentation pattern as an 18S RNA marker. In a CsCl gradient these particles banded like a labeled peak with a buoyant density of 1.54 g/cm3. This peak coincided with that corresponding to the 40S subunits originating from control polysomes, indicating that the overall composition of labeled particles was identical to that of 40S particles from controls. The 40S particles synthesized in the presence of aflatoxin B1 were indistinguishable from their control counterparts in their ability to be incorporated into polysomes and thus to initiate protein synthesis in the rat liver.

197. RIBOSOMES AND CANCER.

Delaunay J, Schapira G
Inst. Mol. Pathol., Paris, France
Biomedicine; 20(5):327-332 1974

Due to the role played by ribosomes in the expression of genetic information, ribosomal structure and function in healthy and carcinoid states was reviewed. The nature of ribosomal binding to the endoplasmic reticulum may be altered in cancer, as indicated by differences in polysome size in healthy and cancerous cells. Ribosomal rRNA may also be different in cancerous cells by containing either more or fewer nucleotides than normal. Ribosomal protein analysis by polyacrylamide gel electrophoresis has shown differences between healthy and cancerous tissues. Actinomycin D and camptothecin are two drugs which, in low concentrations, may be used to selectively block nucleolar synthesis of 45 S RNA, and are thus useful in investigations of rRNA's role in cancer. The vinca alkaloids are also useful due to their ability to inhibit 1' S RNA synthesis in human cells. **Ricin and arbrin, which inhibit tumor growth, and aflatoxin B1, which is a carcinogen, are also useful agents due to their abilities to alter RNA synthesis in different ways.** It is concluded that there is difficulty in establishing causality in the relationship of ribosomes and cancer.

198. CHARACTERIZATION OF MONOSOMES PRODUCED BY AFLATOXIN B1

Hayes LC, Plapp FV, Tilzer LL, Chiga M
Univ. Kansas Medical Center, Kansas City, Kans. 66103
Chem Biol Interact; 10(5):343-347 1975

To elucidate the mechanism by which aflatoxin B1 disaggregates liver polysomes, the monosomes produced by aflatoxin B1 were studied. Male F-344 rats were injected ip with 1.5 mg/kg aflatoxin B1 and sacrificed 18 hr later. The livers were homogenized and fractionated to obtain ribosome pellets from the postmitochondrial supernatant. The 80S monosome peak was isolated to determine the transfer RNA (tRNA) content of the aflatoxin B1-produced monosomes. RNA (350 microg) was layered on polyacrylamide gels, electrophoresed, and the 4S and 5S RNA peaks were traced at 260 nm in a Gilford spectrophotometer. A single dose (1.5 mg) of aflatoxin B1 produced 70% disaggregation of rat liver polysomes into monosomes. After centrifugation, the 80S monosomes dissociated into 40S and 60S ribosomal subunits containing 0.3 M KCl and therefore lacking messenger RNA. The monosomes produced by aflatoxin B1 contained 0.6 molecule of tRNA and/or aminoacyl tRNA but no peptidyl tRNA, 50 similar to monosomes produced by 72-hr starvation. Inhibition of messenger mRNA synthesis could account for decreased numbers of liver polysomes. The observations that the monosomes produced by aflatoxin B1 are dissociated into subunits, and lack peptidyl tRNA indicate that they are runoff ribosomes

199. LATE POLYSOME DISAGGREGATION BY AFLATOXIN B1.

Hayes LC
Univ. Kansas, Kansas Medical Center, Kansas City, KS 66103
Diss Abstr Int B; 38(2):601-B 1977

Experiments were conducted to determine if late polysome disaggregation produced by aflatoxin B1 is the result of inhibition of messenger RNA (mRNA) synthesis via a mechanism involving inhibition of RNA polymerase B or via acid hydrolases. Acute administration of aflatoxin B1 in rats decreased cytoplasmic poly(A)-containing RNA by as much as 50% within 18 hr, while the size distribution of cytoplasmic poly(A)-containing RNA was unaltered. These data provided direct evidence to support the theory that late polysome disaggregation by aflatoxin B' is the result of a decreased quantity of mRNA in the cytoplasm upon which ribosomes can initiate protein synthesis. The characterization of the monosomes resulting from aflatoxin B1 induced late polysome disaggregation supported the theory that the decreased quantity of cytoplasmic mRNA is due to inhibition of synthesis of new mRNA in the nucleus rather than by fragmentation and degradation of pre-existent cytoplasmic mRNA by acid hydrolases. (no Refs)

200. THE EFFECT OF AFLATOXIN B1 ON THE MATURATION OF RIBOSOMAL AND TRANSFER RNA IN MONKEY KIDNEY (CV-1) CELLS IN CULTURE.

Garvican L, Rees KR
Univ. Coll. Hosp. Med. Sch., London, England
Chem Biol Interact; 9(6):429-434 1974

The effects of aflatoxin B1 on the maturation of ribosomal and transfer RNA in monkey kidney (CV-1) cells were studied in vitro. Monolayer cell cultures were labeled with (5-3H)uridine and (2-14C)uridine and incubated with 40 microg/ml of aflatoxin B1. The nucleolar and cytoplasmic RNA were extracted after various chase periods and analyzed by polyacrylamide gel electrophoresis. Aflatoxin B1 inhibited the maturation of rRNA by causing a build-up of 32S RNA in the nucleolus which was associated with a reduced output of 28S rRNA into the cytoplasm. Processing of the 18S rRNA appeared normal. Aflatoxin also delayed the maturation process of tRNA, although the data gave no indication of the point in this process at which the toxin was interfering. The toxin may have interrupted the enzymatic cleavage involved in the maturation process by interaction with the particular RNA involved or by interaction with the enzymes involved in the cleavages. The results also suggest that aflatoxin B1 would be likely to disrupt the maturation process of mRNA.

201. IN VITRO TRANSCRIPTION STUDIES WITH RNA TUMOR VIRUSES. (PP. 281-291)

Jacquet M, Groner Y, Monroy G, Hurwitz J
Fundamental Aspects of Neoplasia. Gottlieb AA, Plescia OJ, Bishop DH, ed. New York, Springer-Verlag, 1975.

The in vitro transcription of viral RNA was examined using myeloblasts from chicks infected with avian myeloblastosis virus (AMV). **RNA products synthesized in vitro by isolated myeloblast nuclei were examined for virus specific sequences by hybridization with complementary DNA (cDNA). RNA synthesized in the presence of a-amanitin did not contain detectable viral RNA,** indicating that the nucleoplasmic RNA polymerase B is probably responsible for the synthesis of AMV RNA sequences. Studies of the hybridization kinetics indicated that viral RNA sequences represent 0.5% of the total RNA in the nuclei. Similar results were obtained when the template used was chromatin isolated from myeloblast nuclei and the reaction was catalyzed by RNA polymerase B. In this case 1% of the total RNA synthesized from chromatin by the eukaryotic enzyme formed RNA:DNA hybrids with DNA. This result, which is higher than expected from a random transcription of the genome, suggests selection transcription of DNA. When purified DNA isolated from myeloblasts was used as template, RNA:DNA hybrid structures were not detected in the transcription product, indicating that hybridizable RNA was specifically in the chromatin-directed transcript. The size of RNA transcripts determined by sedimentation velocity in sodium dodecyl sulfate-sucrose gradients ranged from 5S to 50S. Evidence for initiation of new RNA chains was obtained from studies that showed that a**-32 P-ATP and GTP were incorporated at the 5' termini end of the RNA. Analysis of the a**-32 P-labeled RNA in sucrose gradient revealed that the radioactivity was distributed in products of all sizes, indicating that large RNA moleculaes can be synthesized de novo when chromatin is transcribed by RNA polymerase B. The synthesis of large RNA transcripts from the chromatin suggests that regions of the DNA, which is single-stranded in nature, are used as template. These findings support the hypothesis that the expression of viral information is mediated by the nucleoplasmic enzyme and suggest the possibility that gene activation in eukaryotic organisms results from the unwinding portions of chromatin DNA. (15 refs)

202. OPPOSITE EFFECTS OF PHENOBARBITAL PRETREATMENT ON AFLATOXIN B1 -INDUCED INHIBITION OF TRANSCRIPTION IN RAT AND MOUSE LIVER.

Moule Y, Lesage V, Darracq N, Rousseau N
Inst. Recherches Scientifiques Cancer, Boite Postale no 8, 94800-Villejuif, France
Biochem Pharmacol; 24(20):1851-1854 1975

The action of phenobarbital on the inhibition of RNA synthesis was studied in the livers of aflatoxin-treated rats and mice. Sodium phenobarbital was injected ip (75 mg/kg in 0.2 ml saline) into 16 male Wistar rats for three consecutive days. For 20 male Swiss mice, sodium phenobarbital was either injected ip (75 mg/kg) for 3 days or given as a 0.1% solution in drinking water for at least 9 days. Crystalline aflatoxin B1 (1 mg/kg for rats and 60 mg/kg for mice) dissolved in dimethyl sulfoxide was injected ip three hours before sacrifice. For the determination of in vivo RNA synthesis, 6**-14 Corotic acid was injected ip (5 microCi for rats and 2 microCi for mice) 30 min before sacrifice. RNA in liver nuclei was determined and the radioactivity was measured by liquid scintillation spectrometry. Pretreatment of the rats with phenobarbital decreased the inhibition produced by aflatoxin B1 on in vivo RNA synthesis in the liver nuclei. In mice, however, phenobarbital significantly enhanced this inhibition. The 2 modes of phenobarbital administration to mice gave similar results. Modifications in the morphological nucleolar changes correlated with the biochemical results; phenobarbital decreased the incidence of nucleolar segregation induced in rats by aflatoxin, whereas it produced more pronounced modifications in mouse nucleoli. In rats, the aflatoxin B1 -induced inhibition of RNA polymerase activity was decreased by phenobarbital pretreatment, while in mice it was increased. The data indicate that mouse liver has a potential ability of activating aflatoxin; its resistance is contingent and not determined at the genetic level. It is concluded that repeated ingestion by man of barbituric derivatives combined with a growing number of drugs encountered in the environment must be seriously considered for short- and long-term action.

203. MECHANISM OF THE INHIBITION OF TRANSCRIPTION BY PR TOXIN, A MYCOTOXIN FROM PENICILLIUM ROQUEFORTI.

Moule Y, Jemmali M, Rousseau N
Institut de Recherches Scientifiques sur le Cancer, Boite Postale 8, 94800-Villejuif, France
Chem Biol Interact; 14(3/4):207-216 1976

The biological effects of PR toxin mycotoxin synthesized by Penicillium roqueforti, on the liver of male Wistar rats were studied. The in vivo administration of PR toxin inhibited the in vitro transcriptional capacity of nuclei isolated from the liver. The toxin inhibited the RNA polymerase systems responsible for ribosomal RNA synthesis and heterogenous nuclear RNA synthesis. It did this by preventing the initiation step and the elongation step of transcription.

E. DNA Damage, Synthesis, and Repair Affected by Mycotoxins

204. THE IN VIVO AND IN VITRO EFFECTS OF AFLATOXIN B1 ON RAT LIVER AND SPLEEN ACID DEOXYRIBONUCLEASE.

Pitout MJ, Kemff PG, Schabort JS
South African Med. Res. Counc., Pretoria
Int J Biochem; 5(3):262-270 1974

The in vivo effect of the hepatocarcinogen, aflatoxin B1, on the activity of rat liver acid DNase was studied along with its in vitro effect on rat spleen acid DNase. In the in vivo experiments, aflatoxin B1 (1.5 mg/kg day) was administered intragastrically for 1-7 days to 15 male Wistar-derived rats. In the in vitro experiments, acid DNase was isolated from the spleens of male and female Wistar derived rats, purified, and incubated with aflatoxin B1. The acid DNase in both experiments was analyzed using polyacrylamide gel electrophoresis, electrofocusing, and Sephadex gel-filtration. In the in vivo experiments, aflatoxin B1 dramatically increased the specific activity of the rat liver acid DNase, while in the in vitro experiment, it had no direct activating effect on the splenic enzyme extract. Polyacrylamide gel electrophoresis and electrofocusing revealed that the acid DNase preparation derived from the rat spleens was pure and homogeneous, with a molecular weight of 36,000 and an isoelectric point of 10. With regard to the relationship between aflatoxin B1 and the observed increase in activity of the liver acid DNase, it is suggested that the toxin itself or a metabolite might interact in some way with the activity of the enzyme, possibly via a natural protein inhibitor of the enzyme. It is also possible that the toxin or a metabolic derivative might have a deleterious effect on the lysosomal membranes, thereby resulting in a leakage of acid DNase.

205. THE INFLUENCE OF AFLATOXIN B1 ON NUCLEAR ENDODEOXYRIBONUCLEASE AND DNA OF PRIMARY MONKEY KIDNEY EPITHELIUM TISSUE CULTURE.

Kempff PG, Pitout MJ, Seegers JC
Natl. Res. Inst. Nutr. Dis., Pretoria, South Africa
S Afr J Sci; 70(3):87 1974

Aflatoxin B1 was added to the culture medium of primary monkey kidney epithelial cells grown in tissue culture, at a concentration of 0.1 mg/l medium. The specific activity of nuclear alkaline DNase was slightly elevated after two hr exposure to the aflatoxin B1 but markedly increased after 20 hr exposure. The effect on specific activity of nuclear acid in these experiments was insignificant. In a series of alkaline sucrose gradient centrifugations of these tissue culture cells after administration of aflatoxin B1 at a level of 0.5 mg/l culture medium, DNA breakage was evident in the period, 2-10 hr during exposure but, after this phase, breakages could not be detected. In addition, after exposure of tissue culture cells to aflatoxin B1 for five hr followed by a recovery period of 20 hr (with no toxin in the medium) no DNA breakages could be detected.

206. ON INFLUENCE OF AFLATOXIN B1 ON THE PHYSICAL PROPERTIES OF THE AVIAN MYELOBLASTOSIS VIRUS BAI STRAIN A (AMV) AND ON THE BIOSYNTHESIS OF VIRAL NUCLEIC ACIDS.

Kremen J, Lexova J, Sula J
Med. Fac., Charles Univ., Prague, Czechoslovakia
Neoplasma; 21(3):275-283 1974

The effect of aflatoxin B1 (15 microg/ml) was studied on the physical properties and nucleic acids of avian myeloblastosis virus (AMV) BAI strain A produced by cultured chicken leukemic myeloblasts. Banding of virus on discontinuous sucrose gradients after aflatoxin B1 treatment for 90-320 min showed a change in density from 1.16 g/ml (control) to 1.14 g/ml, similar to that seen in toyocamycin-treated cells. Analysis of virions labeled with 14C-uridine or 32P showed that the 65S viral genomic RNA was absent in aflatoxin B1-treated virus and that only 4S RNA was present. Incorporation of 3H-thymidine into virus-associated 7.5S DNA following synchronization with hydroxyurea was stimulated by about 60% compared with control virions, whereas incorporation into total cellular DNA was inhibited by 58%. It is concluded that synthesis of 65S viral genomic RNA is either completely inhibited or, if synthesized, is rapidly degraded to 4S RNA in aflatoxin-treated virions. The results suggest that 7.5S viral DNA is synthesized independently of 65S RNA or of bulk cellular RNA and DNA.

207. THE EFFECTS OF PROLONGED FEEDING WITH AFLATOXIN B1 ON ADULT RAT LIVER.

Godoy HM, Judah DJ, Arora HL, Neal GE, Jones G
Laboratorio Quimica Biotoxicologica, Zufriategui y Varela, Villa Martelli, Provincia de Buenos Aires, Argentina
Cancer Res; 36(7):2399-2407 1976

The effects of feeding male Fischer rats, weighing approximately 280 g, for 6 wk with a carcinogenic regimen of aflatoxin B1 -contaminated diet are described. A diet of peanut meal was naturally contaminated with aflatoxin B1, the final diet containing 4 ppm. Histological effects of liver sections were related to changes observed in nuclear separations made using zonal centrifugation. Changes in nuclear RNA and DNA synthesis levels were studied in the populations of hepatic nuclei separated in the zonal rotor. The first 3 wk of the feeding period were accompanied by continuing **inhibitions of nucleic acid synthesis,** terminating in a loss of the majority of the tetraploid hepatocyte nuclear population. The subsequent 3 wk of feeding was predominantly a period of proliferation, restoration of the lobular architecture, and recovery of nucleic acid synthesis. The possible bases of these two opposite effects, inhibition followed by stimulation, which occurred sequentially during the continued feeding of the toxic diet, are discussed: the toxin could transform certain of the parenchymal cells into a resistant strain, or a preexisting resistant population of cells could proliferate to take the place of the susceptible population as it necroses.

208. COMPARISON OF HEPATIC CELL KINETICS IN PRECANCEROUS STAGES INDUCED BY SOME HEPATOCARCINOGENS (MEETING ABSTRACT).

Nishizumi M, Albert RE, Burns FJ
New York U. Med. Ctr., N.Y.
Proc Am Assoc Cancer Res; 15:96 1974

This study was designed to compare hepatic cell kinetics in the precancerous stages induced by 2-fluorenylacetamide (2-FAA), aflatoxin B1 and diethylnitrosamine (DEN). 2-FAA (0.03% in diet), aflatoxin B1 (1 ppm in diet) or DEN (20 ppm in drinking water) was administered for 15 wk to Charles River (CD strain) male rats. Extent of the loss of tritium in hepatic DNA labeled at weanling stage and the uptake of a pulse of ^{3}H-TdR into hepatic DNA was substantially greater in the 2-FAA group than in the other 2 groups, though **all 3 experimental groups showed higher uptake of ^{3}H-TdR into hepatic DNA** and higher labeling indices of parenchymal cell nuclei at 8 or 12 wk than in the control group. The labeling index was associated with the severity of the histological changes in hepatic tissue, as hyperplastic nodules were seen in the 2-FAA and 100 ppm DEN groups which revealed labeling indices 6 and 25 times higher than controls respectively at 12 wk. The present results, taken together with our previous work, indicate that higher doses of 2-FAA and DEN enhance hepatic DNA synthesis disproportionately more compared to lower doses, and also suggest that the intensity and time dependence of hepatic cell proliferation differ for different carcinogens at dose rates with comparable T50 values for hepatoma induction.

209. AFLATOXIN B1 (AFB1) DOES NOT PROLONG HEPATIC DNA REPAIR (MEETING ABSTRACT).

Keefe DA, Edwards GS
Dept. Pharmacol., George Washington U., Washington, D.C.
Proc Am Assoc Cancer Res; 15:17 1974

Single doses of the carcinogens DMN, DEN, nitrosomorpholine, nitrosopiperidine, and AAF cause breakage of rat hepatic DNA followed by a prolonged period of slowed repair (Proc AACR 1973, Abs 19, 20, 111). Subsequent reports have shown this to be a characteristic of a wide variety of carcinogens. Noncarcinogenic analogs, alkylators and mutagens may also cause DNA breakage, but this is usually repaired within a few hr. We now report that AFB1 , a potent hepatocarcinogen, causes relatively little DNA breakage in rat liver even at supralethal doses. Single stranded DNA breakage is detectable in alkaline sucrose gradients at 0.5 hr after dosing 2 mg/kg AFB1 (the LD50), is maximal at 2-3 hr and is no longer detectable between 6 and 8 hr. Lower doses produce less breakage, with little or none detectable at 0.1 mg/kg. This breakage could be the result of DNAase activity, which is increased following AFB1 administration, rather than by direct alkylation or arylation. Positive control doses with DMN show extensive DNA breakage and repair is still incomplete at 1 wk. These results provide further evidence that the primary site of AFB1 acute action may not be on the DNA (see Saunders et al, Cancer Res 32: 2487; 1972; Edwards and Allfrey, B. B A 229: 354; 1973).

210. DNA REPAIR IN HUMAN CELLS TREATED WITH ACTIVATED AFLATOXIN B1 (MEETING ABSTRACT).

Sarasin A, Hanawalt P
Dept. Biological Sciences, Stanford Univ., Stanford, CA 94305
Mutat Res; 46(2): 151 1977

The extent of DNA repair in W138 cells was proportional to the aflatoxin concentration up to 40-60 ug/ml but then leveled off at higher concentrations. The repair synthesis continued for over 12 hr after a 20-min treatment with 40 ug/ml aflatoxin B1. About the same extent of DNA repair as in W138 cells was seen in SV40-transformed W138 cells and in CV-1 monkey cells. SV40-transformed Xeroderma pigmentosum (XP) cells, unable to excise pyrimidine dimers, reproducibly exhibited an amount of repair 15-20 fold less than in wild-type cells. The plateau in XP cells occurred at about the same drug concentration as for W138 cells. (7 Refs)

211. REPAIR OF DNA IN HUMAN CELLS AFTER TREATMENT WITH ACTIVATED AFLATOXIN B1.

Sarasin AR, Smith CA, Hanawalt PC
Institut de Recherches Scientifiques sur le Cancer, Boite Postale 8, 94800 Villejuif, France
Cancer Res; 37(6):1786-1793 1977

Repair replication was examined in cultured human cells exposed to the hepatocarcinogen aflatoxin B1 using the combined bromodeoxyuridine density label and radioisotopic label method. Semiconservative DNA synthesis was strongly inhibited, and the repair replication mode was stimulated in diploid fibroblasts (W138) and in their SV40 transformants (VA13) only when exposure to aflatoxin B1 was in the presence of an activating system containing rat liver microsomal enzymes. The maximum amount of repair synthesis was about 20% of that obtained after saturating doses of ultraviolet light (UV). The time course of repair synthesis was similar to that seen after UV, and most of the synthesis was in 30- to 50-nucleotide 'short patches'. A line of SV40-transformed xeroderma pigmentosum cells (Group A) deficient in repair after exposure to UV was similarly deficient in repair replication after aflatoxin treatment. Treatment with aflatoxin resulted in a 25 to 45% inhibition of UV-induced repair replication, suggesting that in addition to producing lesions in DNA, which are substrates for the excision repair system, the toxin also inhibits excision repair. CsCl gradients of DNA treated in vitro with activated aflatoxin demonstrated binding of the drug to DNA. Alkaline sucrose gradient sedimentation gave no indication that single-strand breaks or alkali labile bonds were introduced into DNA by treatment of cells with activated aflatoxin. (41 Refs) (Author Abstract)

212. SIGNIFICANCE OF TREATMENT INTERVAL AND DNA REPAIR IN THE ENHANCEMENT OF VIRAL TRANSFORMATION BY CHEMICAL CARCINOGENS AND MUTAGENS.

Casto BC, Pieczynski WJ, Janosko N, DiPaolo JA
BioLabs, Inc., 2910 MacArthur Blvd., Northbrook, IL 60062
Chem Biol Interact; 13(2):105-125 1976

Treatment of Syrian hamster embryo cells with diverse classes of chemical carcinogens enhanced transformation by a carcinogenic simian adenovirus, SA7. Optimal enhancement was a function of time of chemical addition in relation to time of virus addition and cell transfer. Aflatoxin B1 and the polycyclic hydrocarbons, benzo(a)pyrene, 3-methylcholanthrene, and 7,12-dimethylbenz(a)anthracene enhanced SA7 transformation when added prior to virus, but inhibited transformation when added after virus adsorption and cell transfer. The enhancement of SA7 transformation was maximal when cytosine arabinoside, caffeine and 6-acetoxybenzo(a)pyrene were added after virus, but minimal when these were added before virus. A third class of chemicals, including b-propiolactone, methyl methanesulfonate, N-acetoxy-2-acetylaminofluorene, N-methyl-N'-nitro-N-nitrosoguanidine, and methylazoxymethanol acetate, enhanced SA7 transformation when added before, or after, virus inoculation and cell transfer. All chemicals, which induced changes in DNA sedimentation in alkaline sucrose gradients and unscheduled DNA (repair) synthesis, increased the frequency of SA7 transformation. However, several chemicals such as dibenz(a,h)anthracene, benzo(e)pyrene, cytosine arabinoside, and caffeine enhanced SA7 transformation but did not induce DNA sedimentation changes or repair. Chemicals that cause reparable DNA damage may provide additional sites for integration of viral DNA during the repair process, but those that do not induce DNA repair synthesis may enhance viral transformation by incorporation of viral DNA into sites of unrepaired cell DNA during regular DNA synthesis.

213. THE IDENTIFICATION OF CHEMICAL CARCINOGENS USING RAT LIVER PRIMARY CELL CULTURES FOR THE DETECTION OF DNA REPAIR AND CELL MEDIATED MUTAGENESIS (MEETING ABSTRACT).

San RH, Williams GM
Naylor Dana Inst for Disease Prevention, Valhalla, N.Y. 10595.
Proc Am Assoc Cancer Res; 18:163 1977

The detection of carcinogen-induced DNA repair in rat liver primary cultures (LPC) was described (Williams, Cancer Letters 1:231-235, 1976). It has now been found that a wide variety of procarcinogens including mycotoxins, polycyclic aromatic hydrocarbons, arylamines, nitrosamines and aminoazo dyes will elicit DNA repair in this system, indicating a diverse capability for metabolic activation. Treatment of cultures of adult rat liver (ARL) epithelial lines by members of the same classes of procarcinogens at 10**-6M for 6 weeks resulted in increases in the frequency of 8-azaguanine resistant mutants suggesting low but significant metabolic activation. To enhance the sensitivity, LPC were employed as an activation feeder layer for the target ARL cells. LPC and ARL cells aggregated in close proximity. 24 hr. treatment of these mixed cultures with dimethylnitrosamine resulted in enhanced mutagenesis over that seen with treatment of the ARL cells alone. Further studies of LPC-mediated mutagenesis with other classes of chemical carcinogens will be presented. (Author Abstract)

214. MEASUREMENT OF "UNSCHEDULED" DNA SYNTHESIS IN HELA CELLS BY LIQUID SCINTILLATION COUNTING AFTER CARCINOGEN TREATMENT.

Martin CN, McDermid AC, Garner RC
Cancer Res. Unit, Univ. York, Heslington, York Y01 5DD, England
Cancer Lett (Amsterdam); 2(6):355-360 1977

Liquid scintillation counting was used to detect DNA repair in HeLa cells following treatment with a carcinogen. The HeLa cells were conditioned in a medium deficient in arginine to reduce S-phase DNA synthesis. They were then treated with either N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 7-bromomethylbenz(a)anthracene (BrMBA), N-acetoxy-2-acetylaminofluorene (N-acetoxy-AAF), or ethyl methanesulfonate (EMS). DNA synthesis occurred in all cells treated with a carcinogen, as shown by the incorporation of ^{3}H-thymidine. In a parallel experiment using mouse L cells and N-acetoxy-AAF, no unscheduled DNA synthesis was observed. Aflatoxin B1 induced low levels of repair that were greatly increased by the addition of liver stock solution. The results suggest the usefulness of liquid scintillation in screening for chemical carcinogens. (15 Refs)

215. UNSCHEDULED DNA SYNTHESIS INDUCED IN HEPATOCELLULAR SUSPENSIONS AND PRIMARY CULTURES OF HEPATOCYTES BY PROCARCINOGENS (MEETING ABSTRACT).

Michalopoulos G, Sattler GL, O'Connor D, Pitot HC
Dept of Pathology and Oncology, Univ of Wi, Madison, Wi, 53706.
Proc Am Assoc Cancer Res; 18:246 1977

Unscheduled DNA synthesis was measured in suspensions of freshly isolated adult rat hepatocytes and in 5-day old primary cultures of hepatocytes on floating collagen membranes, as a result of administration of carcinogens and procarcinogens. The hepatocytes were incubated with ^{3}H-thymidine (10 mμCi/ml), hydroxyurea (25 mM) and logarithmically decreasing concentrations of procarcinogens. The DNA was isolated on linear CsCl density gradients. The compounds 2-fluorenylacetamide, benzpyrene, aflatoxin, dimethylnitrosamine, 3'methyl-dimethylaminoazobenzene, and safrole were tested. High concentrations (10**-3M-10**-4M) of the procarcinogens were toxic to the hepatocytes. At lower concentrations (10**-5M-10**-8M) various levels of unscheduled DNA synthesis (from 50% to 300% of the control values) were elicited by these carcinogenic compounds in both primary cultures and suspensions of hepatocytes. These results demonstrate that primary cultures of hepatocytes on collagen membranes can be useful as screening systems for the detection of environmental carcinogens and for studies of in vitro hepatocarcinogenesis. (Author Abstract)

216. THE RESPONSE OF XERODERMA PIGMENTOSUM CELLS AND CONTROLS TO THE ACTIVATED MYCOTOXINS, AFLATOXINS AND STERIGMATOCYSTIN.

Stich HF, Laishes BA
Cancer Res. Centre, Univ. British Columbia, Vancouver, Canada
Int J Cancer; 16(2):266-274 1975

The activation of the mycotoxins aflatoxin B1, G1, B2, G2 , aflatoxicol , and sterigmatocystin by microsomal and supernatant of livers of several species (Syrian hamster, Swiss mouse, New Zealand white rabbit, Muscovite duck, and Rainbow trout) was examined. Cultured fibroblasts of normal persons and of DNA repair-deficient Xeroderma pigmentosum patients were then incubated with these treated fractions to compare their effects. A 9S fraction microsomal preparation of liver (105,000 x g) or (105,000 x g) supernatant of liver was cultured with the fibroblasts. **DNA repair synthesis, chromosome aberrations and clone forming capacity were used as endpoints.** The activation mixtures significantly increased the chromosome breaking function, lethality and DNA damaging effect (measured as DNA repair synthesis) of aflatoxin B1, G1, aflatoxicol, and sterigmatocystin. The DNA repair-deficient XP cells responded to the activated mycotoxins with a low level of unscheduled 3HTdR incorporation as compared to that of control cells, but showed a highly elevated sensitivity to the chromosome-damaging and lethal effect of aflatoxin B1 and sterigmatocystin. The results emphasize the importance of employing activation procedures and of using 'sensitive' human cells to reveal the cytotoxic effects of chemical compounds.

217. THE ROLE OF METABOLIC ACTIVATION OF CHEMICAL PRECARCINOGENS IN THE INDUCTION OF DNA DAMAGE AND REPAIR IN MAMMALIAN CELLS.

Laishes BA
Univ. British Columbia, Vancouver, B.C., Canada
Diss Abstr Int B; 36(7): 3222B-3223B 1976

The relationship between metabolic activation of chemical precarcinogens, DNA damage induced by reactive carcinogenic metabolites, and repair of the damaged DNA was investigated in cultured mammalian cells and in the intact animal. Using 2-acetylaminofluorene (AAF) and its metabolites, the damage induced in cultured fibrobalsts followed the order A-acetoxy-AAF more than N-hydroxy-AAF more than AAF. When the precarcinogens dimethylnitrosoamine, aflatoxin B1, and sterigmatocystin were combined with NADPH-dependent activation systems, their ability to induce high levels of DNA repair synthesis, chromosome aberrations, and cell lethality was increased. Further studies indicated that aflatoxin B1 or sterigmatocystin probably can induce tumors other than those of the liver. Mycotoxins that contained an isolated vinyl ether double bond induced higher levels of DNA repair synthesis than mycotoxins lacking this structure. Studies with 4-nitroquinoline 1-oxide indicated an organ specificity of DNA damage that appeared to correlate with levels of DNA repair synthesis. High levels of DNA damage and repair may result in defective or incomplete repair and neoplastic transformation. (0 Refs)

218. COMPARATIVE EXAMINATION ON INDUCIBILITY OF MUTATION, CHROMOSOME ABERRATION AND DNA-SINGLE-STRAND BREAKAGE OF CULTURED MAMMALIAN CELLS BY APPLICATION OF MYCOTOXINS (MEETING ABSTRACT).

Umeda TM, Tsutsui T, Ithoh M, Saito M
Yokohama City Univ., Sch. Medicine, Yokohama, Japan
Mutat Res; 38(5):351-352 1976

The mutagenic effect of various mycotoxins was measured with a mutation assay in which FM3A cells acquire 8-azaguanine (8-AG) resistance by treatment with mutagenic chemicals. Colonies from the treated cells were grown on agarose plates with or without 20 microg 8-AG/ml. In 8-AG or HAT medium, the cells obtained from large colonies grown on 8-AG plates were ascertained for maintenance of 8-AG resistance during 3 mo cultivation. Aflatoxin B1, mycophenolic acid, patulin, penicillic acid and sterigmatocystin were mutagenic; chetoglobosin B, fusarenon-X, (-) luteoskyrin and ochratoxin A were not mutagenic. The mutagenic mycotoxins were also treated for their capacity to induce chromosomal aberrations and DNA single strand breaks in FM3A cells. Chromosomal aberrations were induced by these mycotoxins; single strand breaks were demonstrated only in cells treated with patulin or penicillic acid and scarcely in cells treated with aflatoxin B1, mycophenolic acid or sterigmatocystin. (no refs)

219. DETECTION OF CARCINOGEN INDUCED DNA DAMAGE BY ALKALINE ELUTION (MEETING ABSTRACT).

Petzold G, Harbach P, Swenberg J
The Upjohn Company, Kalamazoo, Michigan 49001
Proc Am Assoc Cancer Res; 17:31 1976

The alkaline elution technique of Kohn et al (Biochem 13:4134, 1975) has been modified for detecting in vitro and in vivo DNA damage induced by carcinogens. ^{14}C-thymidine labeled V79 cell cultures, in the presence or absence of a rat liver microsomal activation system, were exposed to compounds of known carcinogenic potential. At various time intervals, cells were harvested and the size of DNA was determined using the alkaline elution assay. DNA damage was demonstrated for known carcinogens including: aflatoxin, aromatic amines, ethionine, hydrazines, sulfonates, nitrofuranes, nitrosamines, 4NQO, nitrosoureas and polycyclic hydrocarbons. In general, dose-response curves were obtained for carcinogens not requiring metabolic activation, but were more variable for those requiring it. Several non-carcinogens did not cause DNA damage. In vitro DNA damage was correlated with cell viability at the time of assay and with survival. Additional studies demonstrated in vivo damage to DNA by several known carcinogens. To date, reproducible assays have been developed for liver, kidney, lung, thymus and brain using ^{3}H-thymidine labeled rodents. Methods for additional organs are under development. Maximal damage was detected in tissues most susceptible to oncogenesis. (Author Abstract)

220. IN VITRO DNA DAMAGE/ALKALINE ELUTION ASSAY FOR PREDICTING CARCINOGENIC POTENTIAL (MEETING ABSTRACT).

Swenberg J, Petzold G, Harbach P
The Upjohn Company, Kalamazoo, Michigan, USA 49001
Third International Symposium On Detection And Prevention Of Cancer; 78-79 1976

The ever enlarging list of compounds needing carcinogenic assessment, coupled with limited resources available for long term animal studies necessitates the development of rapid, inexpensive assays for predicting carcinogenic potential. A good correlation has been demonstrated between carcinogenicity and DNA damage in mammalian cell lines. Most studies have utilized alkaline sucrose gradients to demonstrate DNA damage; however, this technique is not well suited for screening. We report the use of an alkaline elution assay to detect DNA damage induced in V79 cells by compounds of known carcinogenic potential. ^{14}C-thymidine labeled cell cultures, in the presence or absence of a rat liver microsomal activation system, were exposed to the chemicals. At various time intervals, cells were harvested, applied to 2.0 micron polyvinyl filters, lysed, and their DNA was eluted from the filters using 0.02M tetrapropylammonium hydroxide, 0.02M Na3EDTA at pH 12.2. The radioactivity of ten 1.5 ml factions and that remaining on the filter was determined. DNA damage has been demonstrated for known carcinogens belonging to the following categories: aflatoxin, aromatic amines, hydrazines, methane sulfonates, nitrofuranes, nitrosamines, nitroquinoline oxide, nitrosoureas, and polycyclic aromatic hydrocarbons. In general, dose-response curves were well established for carcinogens not requiring metabolic activation, but were more variable for those requiring activation. Several non-carcinogens did not cause DNA damage. (Author Abstract)

V. FORMATION OF MYCOTOXINS

221. PRODUCTION OF TRICHOTHECENE MYCOTOXINS BY FUSARIUM SPECIES IN SHAKE CULTURE.

Ueno Y, Sawano M, Ishii K
Tokyo Univ. Science, Ichigaya, Tokyo-162, Japan
Appl Microbiol; 30(1):4-9 1975

Twelve T-2 toxin-producing isolates and 4 fusarenon-X-producing isolates of various Fusarium species were examined for their ability to produce trichothecane mycotoxins on 3 liquid media in shake cultures and jar fermentors. The T-2 toxin producers, such as Fusarium solani, F sporotrichioides, and F tricinctum, produced T-2 toxin and neosolaniol in semisynthetic medium. F solani M-1-1 produced the largest amount of mycotoxins in a nutrient medium consisting of 5% glucose or sucrose, 0.1% peptone, and 0.1% yeast extract in either shake culture or jar fermentation at 24-27 C for 5 days; metal ions did not significantly increase the yield of either mycelium or T-2 toxin. None of the isolates produced significant amounts of fusarenon-X in shake cultures. The data indicate that shake culture on medium containing 5% glucose is suitable for the production of T-2 toxin as well as for screening T-2 toxin producers.

222. ASPERGILLUS FLAVUS AND AFLATOXIN PRODUCTION IN ACID-TREATED MAIZE.

Bothast RJ, Goulden ML, Shotwell OL, Hesseltine CW
Northern Regional Res. Lab., Agricultural Res. Service, U.S. Dept. Agriculture, Peoria, IL 61604
J Stored Products Research; 12(3):177-183 1976

The relationship between aflatoxin formation and Aspergillus flavus was investigated. Isobutyric acid (IBA) and propionic-acetic acid (PA) were applied to comparable lots of freshly harvested yellow dent maize containing 27% moisture. After 6 mo storage, 30% A flavus infection and low levels of aflatoxin were detected in adjacent bins of IBA treated and PA treated maize. Samples were taken after 7 mo from moldy spots in each bin and evaluated for aflatoxin, zearalenone, ochratoxin and microorganisms. A flavus (10^4 propagules/g) was detected in 405 of the PA samples; no aflatoxin was found. Furthermore, counts of A fumigatus, Absidia and Penicillia were high. In addition to the molds found on PA maize, A niger was detected on IBA treated maize; A flavus (10^4 to 10^7 propagules/g) was present in 79% of the IBA samples; aflatoxin (2 to 857 nanog/g) was detected in 57%. Aflatoxin contamination varied between locations within a moldy area. Since toxin-free kernels were often adjacent to highly contaminated ones, bulk quantities of maize must be appraised on the basis of individual kernels. (15 Refs)

223. EFFECT OF INITIAL PH ON AFLATOXIN PRODUCTION.

Buchanan RL, Ayres JC
Dept. Food Science, Univ. Georgia, Athens, Ga. 30602
Appl Microbiol; 30(6):1050-1051 1975

The effect of initial pH on aflatoxin production by Aspergillus parasiticus NRRL 2999 was examined in a semisynthetic medium. Maximal growth, aflatoxin production, and aflatoxin production per unit of growth occurred at initial pH levels of 5.0, 6.0, and 7.0, respectively. Initial pH levels less than pH 6.0 favored production of the B toxins, whereas levels greater than pH 6.0 favored production of the G toxins. The results indicate that initial pH has no universal effect on aflatoxin production.

224. EFFECT OF ZINC ON ADENINE NUCLEOTIDE POOLS IN RELATION TO AFLATOXIN BIOSYNTHESIS IN ASPERGILLUS PARASITICUS.

Gupta SK, Maggon KK, Venkitasubramanian TA
Department of Biochemistry, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, 110007, India
Appl Environ Microbiol; 32(6):753-756 1976

The adenylic acid systems of Aspergillus parasiticus were studied in zinc-replete and zinc-deficient media. The adenosine 5'-triphosphate levels of the fungus were high during exponential phase and low during stationary phase in zinc-replete cultures. On the other hand, the levels of adenosine 5'-diphosphate and adenosine 5'-monosphosphate were low during exponential phase of growth and high during stationary phase. The adenosine 5'-triphosphate levels during exponential phase may indicate higher primary metabolic activity of the fungus. On the other hand, high adenosine 5'-monophosphate levels during stationary phase may inhibit lipid formation and may enhance aflatoxin levels. The inorganic phosphorus content was low in a zinc-replete medium throughout the growth period, thereby favoring aflatoxin biosynthesis. The energy charge during the exponential phase was high but low during the stationary phase. In general the energy charge values were lower because of high adenosine 5'-monophosphate content. (Author Abstract)

225. EFFECTS OF ^{60}CO GAMMA IRRADIATION ON AFLATOXIN B1 AND B2 PRODUCTION BY ASPERGILLUS FLAVUS.

Applegate KL, Chipley JR
Dept. Poultry Sci., Ohio State Univ., Columbus
Mycologia; 46(3):436-445 1974

The effects of ionizing radiation on Aspergillus flavus production of aflatoxin B1 and B2 were studied. Germination of spores of the toxinogenic organism A flavus NRRL-3145, previously exposed to specific gamma radiation levels, resulted in a greater production of aflatoxins B1 and B2 following inoculation onto a medium than did similarly · treated nonirradiated controls. The most notable increases in aflatoxin production occurred from cultures developing from spores having been irradiated with 50, 100, 150, or 200 krad of gamma radiation. Exposure to 400 krad resulted in complete inhibition of spore germination and consequently negative toxin production. Of the two aflatoxins studied, the quantities of B2 toxin produced were significantly lower than that of B1 toxin except in synthetic medium where B2 production was comparable to that of B1 . Analysis of variance revealed that substrate, time of incubation following the respective irradiation doses, as well as radiation levels, all affected the quantities of aflatoxin produced by this organism.

226. INHIBITION OF AFLATOXIN PRODUCTION AND TENTATIVE IDENTIFICATION OF AN AFLATOXIN INTERMEDIATE 'VERSICONAL ACETATE' FROM TREATMENT WITH DICHLORVOS.

Schroeder HW, Cole RJ, Grigsby RD, Hein H
Agr. Res. Serv., U.S. Dept. Agr., College Station, Tex.
Appl Microbiol; 27(2):394-399 1974

Low-aflatoxin-producing (Aspergillus flavus) and high-aflatoxin-producing (A parasiticus) strains of fungus were grown in the presence of the insecticide dichlorvos (0, 5, 10, 15, or 20 microg/ml, w/v basis). Dichlorvos at all four concentrations inhibited aflatoxin B1 production by three isolates of A flavus and one isolate of A parasiticus. With the A parasiticus isolate and two of the A flavus isolates, dichlorvos at 5 microg/ml reduced aflatoxin production approximately 90%; increasing insecticide concentrations decreased aflatoxin yields further but at a sharply reduced rate. Aflatoxin B2 yields were decreased proportionately, as were yields of aflatoxin G1 and G2 by A parasiticus. In a fourth A. Flavus isolate, dichlorvos at the 5 microg/ml level stimulated aflatoxin production, which was inhibited by higher dose levels. The higher insecticide concentrations delayed sporulation in the various isolates. Reductions in the production of aflatoxins were accompanied by the appearance of an orange pigment, the concentration of the pigment increasing in direct proportion to the reduction in aflatoxin production. Spectral analyses of the pigment and its methylated and acetylated derivatives showed it to be versiconal acetate (IV). The data suggest that IV is an intermediate in the metabolic cycle that may terminate in the production of aflatoxin or the versicolorins, or both. Dichlorvos apparently inhibits the biosynthesis of the difurano ring structure common to the aflatoxins and versicolorins.

227. INHIBITION OF GROWTH AND AFLATOXIN PRODUCTION BY CINNAMON AND CLOVE OILS, CINNAMIC ALDEHYDE AND EUGENOL.

Bullerman LB, Lieu FY, Seier SA
Dept. Food Science and Technology, Univ. Nebraska-Lincoln, Lincoln, NB 68583
J Food Sci; 42(4):1107-1109 1977

Previous work indicated that cinnamon was inhibitory to the growth of aflatoxin-producing molds. The objective of this study was to determine the specific components of cinnamon and cloves that may be effective against mold growth and toxin production. The effects of cinnamon oil, clove oil, cinnamic aldehyde and eugenol on growth and aflatoxin production by Aspergillus parasiticus were studied using yeast-extract sucrose broth as the substrate. All four substances inhibited mold growth and subsequent toxin production. Cinnamon and clove oils were inhibitory at 200-250 ppm, cinnamic aldehyde at 150 ppm and eugenol at 125 ppm. Since cinnamic aldehyde and eugenol are the respective major components of cinnamon and clove oils, it was concluded that these are the major active antifungal ingredients of these two essential oils. The inhibitory effect of these substances was judged to be inhibition of growth rather than of toxin production. When growth occurred after a delay, aflatoxin production occurred when the cultures reached secondary metabolism. Given sufficient time, cultures which were inhibited initially, but which subsequently grew, produced toxin levels equivalent to control cultures. Levels of the oils above 250 ppm and of cinnamic aldehyde and eugenol above 200 ppm completely inhibited mold growth, or permitted only a small amount of growth that never reached secondary metabolism and never produced aflatoxins during the time of this study. (Author abstract) (4 Refs)

228. INHIBITION OF AFLATOXIN BIOSYNTHESIS BY 2-MERCAPTOETHANOL (MEETING ABSTRACT).

Gupta SK, Maggon KK
Dept. Biochemistry, V.P. Chest Inst., Univ. Delhi, Delhi, India
Indian J Biochem Biophys; 14(1, Suppl): 54 1977

Aflatoxins are the most potent hepatocarcinogens known and attention has been focussed to find out the mechanism by which aflatoxin formation can be controlled. 2-Mercaptoethanol at a concentration of 1 milliM inhibited aflatoxin formation and at 100 milliM levels the fungal growth was inhibited. When added to the resuspended medium, 2-mercaptoethanol inhibited C14-acetate incorporation into both aflatoxins and neutral lipids, thereby showing that the metabolic block was at an early stage of aflatoxin biosynthesis. The inhibition is probably due to its chelating action with zinc, which is essential for aflatoxin production. Interactions of 2-mercaptoethanol with aflatoxin B1 and G1 resulted in the formation of two new fluorescent compounds. The UV and infra-red spectra of these compounds were very similar to parent aflatoxins. The tentative structures of these new aflatoxins are proposed. (no Refs)

229. INHIBITION OF AFLATOXIN BIOSYNTHESIS BY TOLNAFTATE (MEETING ABSTRACT).

Khan SN, Maggon KK
Dept. Biochemistry, Vallabhbhai Patal Chest Inst., Univ. Delhi, Delhi, India
Indian J Biochem Biophys; 14(1, Suppl): 36 1977

Tolnaftate, an antifungal drug, is widely used to control fungal infections. In the present study, the effect of Tolnaftate on aflatoxin biosynthesis by Aspergillus parasiticus NRRL

3240 was investigated. Tolnaftate changed the morphology of Aspergillus parasiticus to yeast like forms and inhibited aflatoxin formation. The formation of aflatoxin G was totally blocked, thereby showing a metabolic block in the conversion of aflatoxin B to G. The incorporation of 1-C14acetate into aflatoxins was significantly inhibited at a concentration of 1 milliM Tolnaftate (3.074 millig/liter or 3.074 mg/10 ml). Attempts to reverse Tolnaftate inhibition by ZnSO4 were unsuccessful. Further work is in progress to study the mechanism of inhibition by this compound. (no Refs)

230. EFFECT OF GLYCOLYTIC INTERMEDIATES ON AFLATOXIN PRODUCTION (MEETING ABSTRACT).

Sivaswami J
Dept. Biochemistry, Vallabhbhai Patel Inst., Univ. Delhi, Delhi, India
Indian J Biochem Biophys; 14(1, Suppl): 21 1977

The effect of glycolytic intermediates on the biosynthesis of aflatoxins in a resuspension system was studied. Aspergillus parasiticus NRRL 3240 was grown on a synthetic salts medium in the presence and absence of zinc for 4 days at 28C +- 1C. Mycelia were resuspended in phosphate buffer (pH 5.8) containing 1 microg Ci 1-C14acetate and a glycolytic intermediate. Glycolytic intermediates like glucose-6-phosphate, fructose-6-phophate, phosphoenolpyruvate etc, were added in three different concentrations. Aflatoxins were extracted and the incorporation of 1-C14acetate into aflatoxins determined. The results obtained have been discussed. (no Refs)

231. PRODUCTION AND STABILITY OF AFLATOXINS, PENICILLIC ACID AND PATULIN IN SEVERAL SUBSTRATES.

Lieu FY, Bullerman LB
Dept. Food Science and Technology, Univ. Nebraska-Lincoln, Lincoln, NB 68583
J Food Sci; 42(5):1222-1224,1228 1977

Aflatoxins B1 and G1, penicillic acid and patulin were each added to Swiss cheese, bologna and cooked cornmeal. The samples were stored at 5 C for 15 min and 3, 12, 24, 48, 120 and 168 hr before extraction and recovery of the added toxins. Aflatoxins B1 and G1 were stable in the foods during the entire storage period. Patulin and penicillic acid, on the other hand, were undetectable in bologna after 12 and 48 hr storage, respectively. In Swiss cheese, after 168 hr of storage at 5 C, 8% of the original penicillic acid remained. With cooked cornmeal, about 80% of the patulin and penicillic acid were detected after 168 hr of storage at 5 C. Growth and experimental toxin production on Swiss cheese, Mozzarella cheese, bologna, bacon, cooked cornmeal, and English muffins were studied by using known toxinogenic strains of Aspergillus flavus, Penicillium patulum and a Penicillium species incubated at 5, 12 and 25 C for 6, 4 and 2 wk respectively. No growth of A flavus or aflatoxin production was observed at 5 and 12 C on any substrate, but at 25 C, growth and aflatoxin production occurred on Swiss cheese, bologna, cooked cornmeal, Mozzarella cheese and bacon. Patulin and penicillic acid were produced in cooked cornmeal at all three temperatures. No patulin or penicillic acid was found in the other food substrates, except for a small amount of penicillic acid in one of the English muffin samples at 5 C, even though the molds grew extensively on all substrates at all three temperatures. (Author abstract) (32 Refs)

VI. DETECTION OF MYCOTOXINS

A. Analysis of Mycotoxins in Foodstuffs

232. ISOLATION AND TOXICITY OF MOLDS FROM FOODS STORED IN HOMES.

Torrey GS, Marth EH
Dept. Food Science, Univ. Wisconsin-Madison, Madison, WI 53706
J Food Protection; 40(3):187-190 1977

Molds collected from home-stored foods were partially identified, and their potential for mycotoxin production was assessed. Samples were taken from refrigerated and nonrefrigerated food and from refrigerator surfaces. Penicillia (49% of 155 isolates) and aspergilli (38%) were the predominant molds. Aflatoxin (9 isolates), kojic acid (3), ochratoxin A (3) penicillic acid (1), and patulin (1) were detected when culture extracts of isolates were screened for the presence of toxic mold metabolites. Six of the nine cultures that were aflatoxigenic were from nonrefrigerated food (3 from bakery goods, 2 from spices, and 1 from home-canned pumpkin), and three were from refrigerated samples (cheese, cottage cheese, and a cake of compressed yeast). A published study has indicated that Aspergillus flavus and other molds with known oncogenic properties for animals were found more frequently in dwellings, food, and wall surfaces associated with patients suffering from neoplastic diseases than in dwellings of control families not affected by these diseases. A more serious attitude, therefore, should be taken toward the presence of molds in home-stored foods. (22 Refs)

233. A SURVEY OF SOME FOODS FOR AFLATOXINS.

Stanton DW
Chemistry Div., Dept. Scientific and Industrial Res., Auckland, New Zealand
Food Technol Aust; 12(4):25 1977

A small survey of aflatoxin levels in susceptible, locally available foods in Auckland was conducted. Only two samples of peanuts imported from India showed contamination with aflatoxin B1 (5 and 6 ug/kg, respectively). (8 Refs)

234. DETERMINATION OF AFLATOXINS IN FOOD PRODUCTS.

Galea V, Bara A
Institutul oncologic, Cluj-Napoca, Romania
Igiena; 25(2):123-128 1976

Investigations on the presence of aflatoxins (AFTX) in four different categories of food products are reported. Following extraction of the AFTX, qualitative determinations were carried out by thin layer chromatography using silica gel G chromatogram sheets in the developing chamber, and chloroform-methanol (95:5), acetic acid-chloroform (1:9) or acetone-chloroform (1:9) as developing reagents; known AFTX compounds were used as controls. Since no control AFTX M1 was available, the M1 type was identified by its instability and blue fluorescence at exposure to UV light and the Rf=1/2 of that of B1 . AFTX were found in 6/34 samples taken from 8 types of cheese and 1 type of cheese crust. The cheeses used included those with only traces of mold contamination, slight mold contamination, spontaneous mold growth developed under optimum laboratory conditions, and cheese aged in pine-tree bark. AFTX B1 was found in 2/6 samples, B1 and M1 in 1/6, B1 + G1 in 2/6 and B1 + G1 + G2 in 1/6. Among 52 samples taken from 11 types of farinaceous foods, AFTX were present in 8/52; B1 and G1 were found in 2/8 corn-flour samples, B1 was found in 2/8 and B1 + G1 + G2 in 4/8 bread samples kept under laboratory conditions until the spontaneous development of molds. Among 29 samples taken from 9 types of fruits and fruit preparations, B1 AFTX was found in 1 sample of prune mash. No AFTX were found in 17 samples of beer and beer-related products. The authors conclude that under normal conditions of food circulation the risk of contamination with AFTX is minimal. (12 refs)

235. AFLATOXINS IN EGYPTIAN FOODSTUFFS.

Girgis AN, El-Sherif S, Rofael N, Nesheim S
Natl. Organization for Drug Control and Res., Cairo, Egypt
J Assoc Off Anal Chem; 60(3):746-747 1977

Thin-layer chromatographic analyses of fresh and stored Egyptian foodstuffs showed that aflatoxins B1, B2, G1 and G2 were present in 14/42 samples at low levels (3-12 ppb). The highest levels were in cottonseed cake. Analyses by the minicolumn procedure gave positive results for only 5 samples, negative results for 31 samples, and indefinite results for 6 samples. (4 Refs)

236. AFLATOXIN IN TUNISIAN ALEPPO PINE NUTS.

Boutrif E, Jemmali M, Pohland AE, Campbell AD
Institut National de Nutrition et de Technologie Alimentaire, Tunis, Tunisia
J Assoc Off Anal Chem; 60(3):747-748 1977

Aflatoxin levels in contaminated Tunisian Aleppo pine nuts (25/50 samples) varied from 25 to 2,080 ppb for aflatoxin B1 and from 56 to 4,570 ppb for aflatoxin G1. Total aflatoxin reached 7,550 ppb in some samples. A traditional pudding prepared from the contaminated nuts contained 83% of the aflatoxin present in the nuts. (4 Refs)

237. AFLATOXINS IN CALIFORNIA TREE NUTS.

Fuller G, Spooncer WW, King AD, Schade J, Mackey B
Western Regional Res. Lab., Agriculture Res. Service, U.S. Dept. Agriculture, Berkeley, CA 94710
J Am Oil Chem Soc; 54:231A-234A 1977

Surveys of the California walnut and almond crops to determine the incidence of aflatoxins showed av proportions of contaminated nuts from the field to be 1 in 28,250 for walnuts and 1 in 26,500 for almonds. The use of electronic and hand sorting for damage was found to be effective in placing contaminated kernels in the inedible rejects. (14 Refs)

238. STATE OF AFLATOXIN IN GROUNDNUT OIL.

Basappa SC, Murthy VS
Central Food Technological Res. Inst., Mysore, India
J Food Sci Technol; 14(2):57-60 1977

Using centrifugation, ultracentrifugation, membrane filtration, and absorption, it was determined that 60-65% of the aflatoxin in commercial groundnut oil is present in the sedimentable cell debris of the oil. The remaining portion of the toxin may be in the solubilized state. Fuller's earth may be useful for the removal of aflatoxin from groundnut oil. (5 Refs)

239. STUDIES ON AFLATOXIN CONTENT OF GROUNDNUT OIL IN ANDHRA PRADESH WITH REFERENCE TO CLIMATIC CONDITIONS AND SEASONAL VARIATIONS.

Giridhar N. Krishnamurthy GV
CFTRI Experiment Station, Hyderabad-18, India
J Food Sci Technol; 14(2):84-85 1977

The extent of aflatoxin contamination of raw groundnut oil in different regions of Andhra Pradesh during different seasons was investigated. Low toxin content was found in oil samples from dry areas, while samples from the humid coastal areas varied widely in toxin content. Overall the toxin content varied from 0 ppb to 5000 ppb. Factors affecting toxin content are discussed. (8 Refs)

240. HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF AFLATOXINS IN WINES AND OTHER LIQUID PRODUCTS.

Takahashi DM
Food and Drug Admin., 50 United Nations Plaza, San Francisco, CA 94102
J Assoc Off Anal Chem; 60(4):799-804 1977

A modified procedure utilizing high pressure liquid chromatography (HPLC) to analyze the aflatoxin content in wines and other liquids was presented. ODS-bonded or CN-bonded silica was used in the column, and a mobile solvent system of water-methanol-acetonitrile was used. Modifications of the HPLC method involved the use of a preliminary column equipped with a coarse fritted disk to clean up the fluid and a reduction in elution solvent volumes. Of the 53 liquid products tested, only 2 contained no aflatoxin. Of the wines tested, Madeira and other sweet wines produced the greatest background interference; red and white table wines, fruit juices, fish sauce and mirins produced the least. The presence of background interference effectively reduced the detection limit of the HPLC procedure. With appropriate preliminary clean-up, 0.02 microg aflatoxin/liter could be detected in the extracts. (17 Refs)

241. THIN LAYER CHROMATOGRAPHIC DETERMINATION OF AFLATOXIN B1 IN EGGS.

Trucksess MW, Stoloff L, Pons WA, Cucullu AF, Lee LS, Franz AO
Food and Drug Admin., Div. Food Technology, Washington, DC 20204
J Assoc Off Anal Chem; 60(4):795-798 1977

A modified method for the detection of 1 nanog/g or less of aflatoxin B1 in hen eggs is presented. Hexane was used as a delipidizer and the delipidized extract was analyzed on a cellulose column and by one-dimensional thin layer chromatography. To test the method, hens were fed contaminated feed, or eggs were injected with aflatoxin and the egg material was assayed. Compounds that were found to interfere with the assay were removed from the eggs. When the aflatoxin was removed from the egg immediately after the egg was injected, a 75% recovery was obtained. When the aflatoxin was extracted 24 hr after injection, a 30% recovery was noted. The addition of sodium chloride or sodium sulfate to the extract eliminated this time-dependent loss of recovery efficiency. It was thought that sodium chloride affected the release of aflatoxin from the egg protein to which it was bound. (8 Refs)

242. DETERMINATION OF PATULIN IN FOODSTUFFS. PART I: DETERMINATION OF PATULIN IN APPLE JUICE.

Polzhofer K
Unilever Forschungsgesellschaft mbH, Behringstrasse 154, D-2000 Hamburg 50, W. Germany
Z Lebensm Unters Forsch; 163(3):183-185 1977

An analytical method was developed permitting the detection of 50 ug of the carcinogen patulin per kg apple juice. After a prepurification of the crude extract by liquid-liquid extraction and column chromatography, quantitative determination of patulin resulted during remission measurements with a chromatogram-spectrophotometer at 273 nanometers. The recovery rates of additions between 120-200 ug patulin/kg were between 82 and 90%. (13 Refs)

243. THE PRESENCE AND CONCENTRATION OF AFLATOXIN M1 IN MILK SHIPPED TO A DAIRY PLANT.

Kiermeier F, Weiss G, Behringer G, Miller M, Ranfft K
Suddeutsche Versuchs- und Forschungsanstalt fur Milchwirtschaft, Vottinger Strasse 45, D-8050 Freising - Weihenstephan, W. Germany
Z Lebensm Unters Forsch; 163(3):171-174 1977

Thin-layer chromatography and mass spectrometry established the presence of aflatoxin M1 in 79/419 milk samples. Of these 79 samples, 0.05-0.1 ug/liter was found in 33% and greater than or equal to 0.1 ug/liter in 38% (av 0.12 ug/liter). The other aflatoxins were absent. A total of 43% of the feed samples taken from dairy farms with aflatoxin M1-positive milk were positive for aflatoxin B1. It was not possible to establish a quantitative correlation between aflatoxin M1 concentration in milk and aflatoxin B1 concentration in feed. (12 Refs)

244. INFLUENCE OF RAW MILK PROCESSING ON THE AFLATOXIN M1 CONTENT OF MILK PRODUCTS.

Kiermeier F, Mashaley R
Lehrstuhl fur Milchwissenschaft der Technischen Universitat Munchen, D-8050, Freising-Weihenstephan, W. Germany
Z Lebensm Unters Forsch; 164(3):183-187 1977

The influence of raw milk processing on the aflatoxin content of milk products is reviewed. When raw or pasteurized milk was stored at 5 C for 1-3 days, there was an 11-25% decrease in the aflatoxin M1 content regardless of natural or artifical contamination. This decrease in aflatoxin content was at first attributed to oxidation, but repeated experiments with exposure to light revealed no connection between oxidation and aflatoxin content. When naturally contaminated milk was freeze-dried, the loss in aflatoxin content was less pronounced than in artificially contaminated milk. When artificially contaminated milk was heated to 71 C for 40 sec, there was a 28.9% decrease in aflatoxin content. Naturally contaminated milk heated to 71 C for 40 sec showed a 13.8% decrease in aflatoxin content. Artificially contaminated milk at 62 C for 30 min showed a 35.2% decrease in aflatoxin content. Artificially contaminated milk at 120 C for 15 min showed a 22.2% decrease in aflatoxin content. Separation of the cream from contaminated whole milk showed 84% of the aflatoxin was in the skimmed milk and 16% was in the cream. When making butter from naturally contaminated cream, 18-28% of the aflatoxin content of the whole milk was found in the butter, while the buttermilk contained the majority of the aflatoxin. The aflatoxin-M1 content increases with decreasing fat levels in dry milk products. (32 Refs)

245. INFLUENCE OF COOL TEMPERATURES ON AFLATOXIN FORMATION IN MILK PRODUCTS.

Kiermeier F, Behringer G
Chemisches und Physikalisches Institut der Suddeutschen Versuchsund Forschungsanstalt fur Milchwirtschaft Weihenstephan der Technischen Universitat Munchen, Vottinger Strasse 45, D-8050 Weihenstephan, W. Germany
Z Lebensm Unters Forsch; 164(4):283-285 1977

Results of experiments with pastes of milk and cheese powder artificially contaminated with Aspergillus parasiticus incubated at 1 C, 5 C, 10 C, and 20 C for 14 days at a relative humidity of 90-95% are discussed in relation to aflatoxin development. At temperatures between 1-5 C the B1, B2, G1, G2, and M1 aflatoxins were still quantitatively measurable. On the av there was a fourfold increase in the concentration of each aflatoxin between 1-5 C in moist milk powder. For aflatoxin M1 at 1 C the concentration went from 5.9 microg/kg on day 0 to 10 microg/kg on day 14; at 5 C it went from 5.9 microg/kg to 12 microg/kg; at 10 C it went from 5.9 microg/kg to 5.5 microg/kg; and at 20 C it went from 5.9 microg/kg to 1156 microg/kg. A short exposure (1-3 days) to higher temperatures (20 C) enables the aflatoxin-producing fungus to establish a strong foothold. The lactose content of the milk or cheese powder had no significant influence on aflatoxin values. Storage of cottage cheese and camembert in a 10% salt solution did not inhibit aflatoxin formation at 20 C. (17 Refs)

246. DETERMINATION OF AFLATOXIN IN MILK AND MILK PRODUCTS.

Polzhofer K
Unilever Forschungsgesellschaft mbH, Behringstrasse 154, D-2000 Hamburg 50, W. Germany
Z Lebensm Unters Forsch; 163(3):175-177 1977

In several milk, milk powder, yoghurt, Camembert, and fresh, hard, and processed cheese samples investigated for possible contents of aflatoxin B1, B2, G1 and M1, only aflatoxin M1 was found. Its av and max values were as follows: 0.07 and 0.33 ug/liter (milk), 0.50 and 2.0 ug/kg (milk powder), 0.20 and 0.47 ug/kg (yoghurt), 0.23 and 0.51 ug/kg (fresh cheese), 0.43 and 1.3 ug/kg (hard cheese), 0.26 and 0.55 ug/kg (processed cheese) and 0.31 and 0.73 ug/kg (Camembert). In the winter, the concentration of aflatoxin M1 in milk was 0.33 ug/liter, compared to a summer value of 0.28 ug/liter. (19 Refs)

247. MILK AFLATOXIN AND TOXICITY TRANSFER.

Ferrando R, Parodi A, Henry N, Delort-Laval J, N'Diaye AL
Laboratoire de Nutrition et d'Alimentation, Ecole nationale Veterinaire, 94701 Alfort
C R Acad Sci D (Paris); 284(10):855-858 1977

Lactating goats were fed three types of peanuts containing 1,530, 79, and 54 ug/kg total aflatoxins and 1,136, 64, and 54 ug/kg aflatoxin B1, respectively, and their milk was lyophilized. In the group consuming peanuts with the highest concentration of total aflatoxin, the dried milk products contained 0.5 ug/kg aflatoxin B1, traces of aflatoxin B2, and 16.2 ug/kg of aflatoxin M1. Milk from all other experimental groups showed no presence of mycotoxin. These dried milk products were given as 20% of their total food ration, to ducklings on days 1 to 23. No adverse effect on growth, feeding, or liver or kidney histology was noted. In contrast, direct consumption by the ducklings of peanut meal with the highest levels of aflatoxin produced an 18% mortality rate and characteristic lesions of aflatoxicosis. (3 Refs)

248. THE PRESENCE AND CONCENTRATION OF AFLATOXIN M1 IN COMMERCIAL CHEESE SAMPLES.

Kiermeier F, Weiss G, Behringer G, Miller M
Suddeutsche Versuchs- und Forschungsanstalt fur Milchwirtschaft, Vottinger Strasse 45, 5050 Freising-Weihenstephan, W. Germany
Lebensmittel Unters Forsch; 163(4):268-271 1977

Thin-layer chromatography and mass spectrometry showed that of 197 cheese samples (approx 20 different varieties), 136 were aflatoxin M1 (AFM1)-positive. The max level was 0.23 ug/kg, and the av level was 0.09 ug/kg. Of these positive samples, 54% showed only traces of AFM1, 21% up to 0.1 ug/kg and 14% greater than 0.1 ug/kg. Soft cheese had less AFM1 than the other varieties. Supplemental spring feeding of cows increased the AFM1 concentration in the cheeses, particularly the soft types. (15 Refs)

249. MYCOTOXINS. SURVEY OF 1975 WHEAT AND SOYBEANS FOR AFLATOXIN, ZEARALENONE, AND OCHRATOXIN.

Shotwell OL, Goulden ML, Bennett GA, Plattner RD, Hesseltine CW
U.S. Dept. Agriculture, Agricultural Res. Service, Northern Regional Res. Center, Peoria, IL 61604
J Assoc Off Anal Chem; 60(4):778-783 1977

The Eppley multitoxin screening method was used in a survey of aflatoxin, zearalenone, and ochratoxin content in wheat and soybean crops from different areas of the country. Wheat samples were collected from Southeastern Missouri, North Carolina, Virginia, Southern Illinois, and Kentucky. Soybean samples came from Nebraska, Minnesota, Iowa, Illinois, Virginia, Alabama, Arkansas, and Texas. None of the three mycotoxins tested were detected in the soybean crops tested. Zearalenone was detected in 19/42 wheat samples collected in Virginia in concentrations ranging from 0.36 to 11.05 ppm. Aflatoxin and ochratoxin were not detected in these wheat samples. Positive identification of zearalenone was achieved through gas-liquid chromatography and mass spectroscopy. In all of the zearalenone-positive samples, Giberella zea infection was detected. Of the kernels in the samples tested, 6-60% contained G zea. (12 Refs)

250. MYCOTOXINS. AFLATOXIN IN CORN: EVALUATION OF FILTER FLUOROMETER READING OF MINICOLUMNS.

Shotwell OL, Goulden ML, Kwolek WF
U.S. Dept. of Agriculture, Agricultural Res. Service, Northern Regional Res. Center, Peoria, IL 61604
J Assoc Off Anal Chem; 60(5):1220-1222 1977

A minicolumn method using a filter fluorometer for determining aflatoxin in corn was compared with thin layer chromatography. More samples were identified as aflatoxin-positive with the minicolumn-fluorometer method than with chromatography method, and the levels of aflatoxin detected showed lack of agreement. The use of the filter fluorometer did not appear to have advantages over visual comparisons of unknowns and standards. (11 Refs)

251. IMPROVED PROCEDURE FOR QUANTITATIVE DETERMINATION OF AFLATOXINS IN CORN AND WET MILLED CORN PRODUCTS.

Barabolak R
Moffett Technical Center, CPC International, Incorporated, Argo, IL 60501
J Assoc Off Anal Chem; 60(2):308-310 1977

A procedure is described for extracting aflatoxins from a variety of agricultural products with aqueous acetone; ammonium sulfate is used to precipitate extraneous interfering substances in minicolumn screening techniques. (8 Refs)

252. AFLATOXIN IN CORN: AMMONIA INACTIVATION AND BIOASSAY WITH RAINBOW TROUT.

Brekke OL, Sinnhuber RO, Peplinski AJ, Wales JH, Putnam GB, Lee DJ, Ciegler A
Northern Regional Res. Center, Agricultural Res. Service, Peoria, IL 61604
Appl Environ Microbiol; 34(1):34-37 1977

Four samples of corn were compared with respect to their hepatocarcinogenicity in rainbow trout. One corn sample was found by chemical analysis to contain no detectable aflatoxin. A second sample was contaminated with aflatoxins at a level of 180 microg/kg. Each of the above-mentioned samples was divided, and 1/2 of each was ammoniated. These four samples were added to a semipurified basal diet and fed to a sensitive strain of rainbow trout. It was found that ammoniation inactivated the aflatoxins and reduced the carcinogenicity of the contaminated corn to a level that was not significantly different from that with the basal control diet. It was also found that the ammoniation process did not reduce the nutritive value of the corn. (Author abstract) (13 Refs)

253. AFLATOXIN CONTAMINATION, FLUORESCENCE, AND INSECT DAMAGE IN CORN INFECTED WITH ASPERGILLUS FLAVUS BEFORE HARVEST.

Lillehoj EB, Kwolek WF, Peterson RE, Shotwell OL, Hesseltine CW
Northern Regional Res. Lab., Agricultural Res. Service, U. S. Dept. Agriculture, Peoria, IL 61604
Cereal Chem; 53(4):505-512 1976

Aspergillus flavus infection of corn ears in South Carolina fields before harvest was identified and recorded photographically; the presence of the fungus was generally associated with insect activity. A flavus-infection fluorescence were related to the occurrence of aflatoxin B1 in corn samples. Fungus-contaminated kernels routinely exhibited two types of fluorescence: bright greenish-yellow in the germ margin, or intense yellow throughout the endosperm. The patterns of fluorescence may either reflect the extent of A flavus invasion or represent products at the initial site of fungal infection.

254. REPORT OF THE THIRD INTERNATIONAL SYMPOSIUM ON MYCOTOXINS HELD IN PARIS ON 16-18 SEPTEMBER 1976.

Van Veldhuizen CJ
Food Inspection Dept., Rotterdam, Netherlands
Voeding; 10(23):8-9 1977

The legal limits for aflatoxins and similar compounds contained in grains and oil-bearing seeds in various countries, methods of sampling and analysis, new mycotoxins, prevention of contamination, detoxification and elimination procedures, and the need for inspection of human food and animal fodder are reported briefly. (no Refs)

B. Analytical Methods for the Detection of Mycotoxins

255. A MODIFIED VELASCO'S METHOD TO DETERMINE AFLATOXIN B1 IN AGRICULTURAL PRODUCTS.

Lu FJ, Ling KH
J Formosan Med Assoc; 73:723-728 1974

In the recent research, we have found out some disadvantages of Velasco's method to determine aflatoxin B1 in agricultural products. Owing to the disadvantages, we have modified the Velasco's method and designed a set of experimental procedures to measure aflatoxin B1. The details of procedures and the advantages of the modified method were discussed. (Author Abstract)

256. NONDESTRUCTIVE DISTINCTION BETWEEN AFLATOXIN B1 AND ETHOXYQUIN IN THIN-LAYER CHROMATOGRAPHY.

Issaq HJ, Barr EW, Zielinski WL
NCI Frederick Cancer Research Center, P.O. Box B, Frederick, Md. 21701
J Chromatogr; 132(1):115-120 1977

A rapid and simple method has been developed for the nondestructive distinction between aflatoxin B1 and the feed antioxidant, ethoxyquin. These two chemicals exhibit similar R subF values in certain solvent systems and produce a similar bluish fluorescence under long UV (366 nm) radiation. The method involves the in situ generation of fluorescence spectra of the respective thin-layer chromatography spots. Since it is nondestructive, the method affords ancillary study of the separated aflatoxins. (Author Abstract)

257. PHARMACOKINETIC STUDIES ON THE HEPATOTOXICITY OF LUTEOSKYRIN. (III) QUANTITATIVE DETERMINATION OF LUTEOSKYRIN IN THE LIVER.

Ueno I
Inst. Medical Science, Univ. Tokyo, Tokyo 108, Japan
Jpn J Pharmacol; 26(1):1-5 1976

A method for the quantitative determination of luteoskyrin in mouse liver is presented. Luteoskyrin was extracted with ether from the liver homogenate after acidification with HCl, purified by thin-layer chromatography, and determined by spectrophotometry. Five microg of luteoskyrin added to 1 g of wet liver, resulted in a recovery rate of 97% and 1 microg, under the same conditions, with a recovery rate of 70%. Luteoskyrin in the amount of 0.5 microg could be detected on the chromatogram, but could not be determined quantitatively. A radioactivity corresponding to 0.92 microg/g tissue of H3-luteoskyrin in the livers from the mice injected iv with this compound was extracted with ether with a recovery rate of 93% and separated on the thin-layer chromatogram with a recovery rate of 81%. The lower limit of quantitative determination of luteoskyrin in 1 gram of liver was 1 microg. Amounts of luteoskyrin in the livers of mice poisoned with this mycotoxin were estimated. Luteoskyrin was recovered in a large quantity in livers from male mice while detection in the liver of females was nil. These findings may correlate with differences according to sex in the susceptibility of mice to hepatotoxicity and hepatocarcinogenicity of this mycotoxin. (10 refs)

258. USE OF WATER SLURRIES IN AFLATOXIN ANALYSIS.

Velasco J, Morris SL
U.S. Dept. Agriculture, Agricultural Res. Service,
Agricultural Marketing Res. Inst., Beltsville, Md. 20705
J Agric Food Chem; 24(1):86-88 1976

A method based on the use of water slurries was developed for aflatoxin analysis. This procedure enables aliquots, from kilogram size samples, to be analyzed without an increase in solvent or reagent costs. Slurries were prepared in a blender, and sample weight to water volume ratios were 1:1.5 for peanut butter, 1:2.25 for whole seeds, 1:4 for commercial meals, and 1:5 for copra. Extraction of 100-g aliquots of slurry with 200 ml of acetone resulted in aflatoxin yields comparable to those obtained by extraction of the dry products by the standard method. Peanuts, peanut butter, peanut meal, cottonseed, cottonseed meal, copra, and corn were successfully analyzed by this slurry technique. The precision of slurry analysis for these products, expressed as coefficient of variation (percent), ranged from 2.6-7.8 compared to 5.7-20.8 for analyses by the standard procedure. Determinations were made by fluorometric measurements of aflatoxin adsorbed on the Florisil layer in a minicolumn. The simplicity and economical aspects of water slurries should enable larger size samples to be analyzed for aflatoxin. This will help reduce the risk of incorrectly assessing the contamination level of sample lots in commercial markets.

259. IDENTIFICATION AND SEMIQUANTITATIVE DETERMINATION OF OCHRATOXINS A AND B, STERIGMATOCYSTIN AND AFLATOXIN B1 .

Lemieszek-Chodorowska K
Zaklad Higieny, 00-791 Warsaw, ul. Chocimska 24, Poland
Rocz Panstw Zakl Hig; 27(2):153-160 1976

One-dimensional thin-layer chromatography was used for the identification and semiquantitative determination of aflatoxin B1 , sterigmatocystin, and ochratoxins A and B isolated in wheat, rye, and rice. The extracts were purified with the use of chromatographic columns filled with celite (for the ochratoxins) or silica gel (for aflatoxin B1 and sterigmatocystin). The purified extracts were developed on the same chromatogram with a benzene + methanol + acetic acid (90 + 5 + 5) solvent system. Mycotoxin fluorescence was evaluated at a wavelength of 366 nm. Sterigmatocystin was visualized by spraying the chromatogram with an ethanol solution of AlCl3 , and an ethanol-water solution of NaHCO3 was used as a spray to increase ochratoxin fluorescence. None of the mycotoxins were found in control tests. In approximately 74 trials fortified with the mycotoxins, mean values in the range 89.5%-92.9% were obtained. The Rf values obtained were: aflatoxin B1 0.25-0.28; ochratoxin B 0.30-0.33; ochratoxin A 0.40-0.43; and sterigmatocystin 0.56-0.59. The lowest detectable amounts in the grain products were 0.001 ug for aflatoxin B1 , 0.0025 ug for ochratoxin A, 0.0025 ug for ochratoxin B, and 0.005 ug for sterigmatocystin.

260. HIGH-PRESSURE LIQUID CHROMATOGRAPHIC METHOD FOR DETECTION AND RESOLUTION OF RUBRATOXIN, AFLATOXIN, AND OTHER MYCOTOXINS.

Engstrom GW, Richard JL, Cysewski SJ
Natl. Animal Disease Center, Veterinary Res. Service, U.S.
Dept. Agriculture, Ames, IA 50010
J Agric Food Chem; 25(4):833-836 1977

The development of a high-pressure liquid chromatography system (HPLC) for the separation of a mixture of seven mycotoxins (aflatoxin B-1, aflatoxin G-1 less than ochratoxin A, zearalenone, rubratoxin B, patulin, and penicillic acid) is reported. The HPLC system included a 254 nanom UV detector, a reverse-phase micron Bondapak/C-18 column and a solvent system made up of acetonitrile, water, and acetic acid (55:45:2, by vol). Standard samples of the mycotoxins were dissolved in ethyl acetate or acetonitrile to give concentrations of 100, 10, 5 and 1 nanog/microliter. Detection limits for the various mycotoxins were 25 nanog for aflatoxin G1 or B1, 1 nanog for zearalenone, 5 nanog for rubratoxin B, and 0.04 nanog for ochratoxin B-1 or G-1. The system also resolved sterigmatocystin from the other mycotoxins in the absence of zearalenone, trichothecin from the others in the absence of ochratoxin A, and roseotoxin B in the absence of rubratoxin B. To detect aflatoxins B-1 and G-1 it was necessary to change the UV filter to 365 nanom. (42 Refs)

261. AFLATOXIN LOCALIZATION BY THE ENZYME-LINKED IMMUNOCYTOCHEMICAL TECHNIQUE.

Lawellin DW, Grand DW, Joyce BK
Dept. Microbiology, Colorado State Univ., Fort Collins,
CO 80532
Appl Environ Microbiol; 34(1):88-93 1977

Visualization of aflatoxin deposits within Aspergillus parasiticus ATCC 15517 hyphae was made possible by using an enzyme-linked immunocytochemical technique. (Author abstract) (4 Refs)

262. ENZYME-LINKED IMMUNOSORBENT ANALYSIS FOR AFLATOXIN B1.

Lawellin DW, Grant DW, Joyce BK
Dept. Microbiology, Colorado State Univ., Fort Collins,
CO 80523
Appl Environ Microbiol; 34(1):94-96 1977

An enzyme-linked immunosorbent analysis permitted the detection of less than 10 picog of aflatoxin B1 per ml. The antitoxin was most specific for aflatoxins B1 and B2(a), and least specific for aflatoxin G1. (Author abstract) (6 Refs)

263. A CLEAN-UP PROCEDURE FOR HPLC ANALYSIS OF AFLATOXINS IN AGRICULTURAL COMMODITIES.

Lansden JA
National Peanut Res. Lab., U.S. Dept. Agriculture, Agriculture Res. Service, Southern Region, Georgia-South Carolina Area, Dawson, GA 31742
J Agric Food Chem; 25(4):969-971 1977

A minicolumn procedure used to qualitatively determine total aflatoxin in peanuts and rice, has been modified to provide clean-up of aflatoxin extracts for high-pressure liquid chromatography. A heavy metal (Zn) precipitation and an alumina filtration were used. (7 Refs)

264. REVERSE PHASE ANALYTICAL AND PREPARATIVE HIGH PRESSURE LIQUID CHROMATOGRAPHY OF AFLATOXINS.

Stubblefield RD, Shotwell OL
U. S. Dept. Agriculture, Agricultural Res. Service, Northern Regional Res. Center, Peoria, IL 61604
J Assoc Off Anal Chem; 60(4):784-790 1977

Reverse high pressure liquid chromatography (HPLC) was used to determine successfully low levels of the aflatoxins B1, B2, G1, G2, M1 and M2 in corn extracts. The isolation and purification of aflatoxins B1, G1 and M1 were also achieved by reverse phase preparative HPLC on a C18 (10 microm, 4 mm inside diameter x 30 cm) column with an acetonitrile-water elution system with a flow rate of 1.5 ml/min. UV absorbance at 350 nanom was used to detect the compounds. HPLC determinations of aflatoxin B1 added to uncontaminated corn extracts at 20 and 50 ppm concentrations were 83 +- 14%, and 92.5 +- 8%, respectively, of the expected levels. HPLC values av approx 25% less than those obtained by thin layer chromatography (TLC). Preparative separations were achieved with a C18/Porasil B (35-75 microm) column (3/8 inch outside diameter x 8 inches) with an acetonitrile-water elution mixture. The isolation of 40 mg of aflatoxin B1 or G1 was accomplished in less than 3 hr with these columns. (18 Refs)

VII. CHEMICAL STUDIES ON MYCOTOXINS

265. REACTION OF COUMARIN WITH AQUA AMMONIA. IMPLICATIONS IN DETOXIFICATION OF AFLATOXIN.

Bergot BJ, Masri MS
Western Regional Res. Lab., Agricultural Res. Service, U.S. Dept. Agriculture, Albany, CA 94710
J Agric Food Chem; 25(4):965-966 1977

One of the systems previously shown to detoxify aflatoxin-contaminated oil seed meal was used for coumarin. A water-soluble beta-amino acid was produced. A similar Michael addition may occur in the detoxification of aflatoxin-containing feeds. (10 Refs)

266. AMMONIUM HYDROXIDE TREATMENT OF AFLATOXIN B1. SOME CHEMICAL CHARACTERISTICS AND BIOLOGICAL EFFECTS.

Vesonder RF, Bechwith AC, Ciegler A, Dimler RJ
Agric. Res. Serv., U. S Dep. Agric., Peoria, Ill.
J Agric Food Chem; 23(2):242-243 1975

Some chemical and biological properties of the reaction product(s) of 8-18-day incubations of aflatoxin B1 with 17 N ammonium hydroxide at room temperature are reported. Thin layer chromatography and infrared and UV emission spectroscopy indicated only aflatoxin B1 to be present after 21 hr incubation. However, when the reaction mixture was taken to dryness after 18 days of incubation, approximately 50% of the solid isolated was a mixture of aflatoxin B1 and of the substituted O-coumaric acid , and the other 50% was a brown, acetone -insoluble solid which was soluble in water and methanol . This residue absorbed at 265 nm in UV and had a broad plateau from 300-362 nm; when excited at 360 nm it fluoresced at 445 nm. When tested for toxicity on fertile white Leghorn eggs, no mortality or teratogenic effects were observed after 20 days at levels of 0.31, 0.031, and 0.005 microg per egg.

267. BIOLOGICAL ACTIVITY AND ELECTRONIC STRUCTURE OF THE AFLATOXINS.

Heathcote JG, Hibbert JR
The University, Salford, England
Br J Cancer; 29(6):470-476 1974

The electronic structures of versicolorin A and aflatoxins B1, G1, and 4-20 dehydro B1 were determined via the simple Huckel molecular orbital method using a computer, to see whether there is a correlation between electronic structure and biological activity in these compounds. The molecular orbital calculations were expressed in terms of the charge density, the free valency of each atom in the molecule, and bond order of pi-electrons of the interatomic bonds. The calculations showed that the 2-3 pi-bond, which has the highest bond order of the aflatoxin molecules, should be the most susceptible to electrophilic attack and is the most probable location of the K region of the molecule. This is in agreement with the previous observation that aflatoxins B1 and G1 hydrate rapidly in dilute acid to the hydroxyaflatoxins B2a and G2a with an apparent total loss of carcinogenicity. The calculations also showed that aflatoxins B1, G1, and M1 have no suitable site for an L region, which probably accounts for their highly carcinogenic nature. Versicolorin A probably has an L region in the anthraquinone portion of the molecule. These data provide the theoretical basis, in molecular orbital terms, for a correlation between electronic structure and carcinogenic activity in the aflatoxins.

268. THE FATE OF AFLATOXINS IN THE PREPARATION OF PROTEIN CONCENTRATES AND ISOLATES FROM CONTAMINATED PEANUT AND COTTONSEED FLOURS.

Stoloff L, Trucksess M, Martinez W
Bureau Foods, Food and Drug Admin., Washington, DC 20204
J Food Sci; 41(5):1251-1253 1976

Simulated commercial protein concentrate and isolate processes were applied to aflatoxin-contaminated peanut and cottonseed flours to determine the fate of the aflatoxins. Processes for producing concentrate by washing the flour at the protein isoelectric point (pH 4.5) were ineffective in separating the aflatoxin from the products, but a process using an aqueous alcohol wash accomplished a 90% reduction in the aflatoxin level of the concentrate compared to the flour. The processes for producing isolate by protein dissolution in alkali and its recovery by precipitation at the isoelectric point resulted in some aflatoxin loss, but there was an increase in the aflatoxin concentration associated with the protein isolate compared to the original flour. However, a process applying carbon adsorbent to the alkaline solution reduced the aflatoxin level of the isolate by 90%. The results show that the basic process used by industry to prepare protein isolates from soybeans will concentrate most of any aflatoxin present in feed stock in the isolate. (10 refs)

269. A NEW ROUTE TO 3A,8A-DIHYDROFURO(2,3-B)BENZOFURANS.

Pawlowski NE, Jones DJ, Sinnhuber RO
Dept. Food Sci. Technol., Oregon St. U., Corvallis
Tetrahedron Lett; (14):1321-1323 1974

The alkylation of phenol in glyme with 3-bromo-1,5-hexadiene in the presence of potassium carbonate yields a primary ether (3) containing less than 1% of its secondary isomer. The borontrichloride catalyzed Claisen rearrangement of the cold ether 3 leads to 0-(3-hexa-1,5-dienyl)phenol (4), in 49% yield. The only side product from the rearrangement is the ether cleavage product, phenol. Oxidative cleavage of the two double bonds in 4 with osmium tetroxide sodium periodate yields a hemiacetal (6,R=OH), which is easily converted to its more stable acetate (7,R=COCH3). **Pyrolysis of 7 yields 3a,8a-dihyrdofuro-(2,3-b)benzofurans, which are intermediates in the formation of aflatoxin B1.**

270. AFLATOXIN B-OXIDE GENERATED BY CHEMICAL OR ENZYMIC OXIDATION OF AFLATOXIN B1 CAUSES GUANINE SUBSTITUTION IN NUCLEIC ACIDS.

Martin CN, Garner RC
Cancer Res. Unit, Univ. York, Heslington, Yorkshire, England
Nature (Lond); 267(5614):863-865 1977

The generation of aflatoxin B1 8,9-oxide by the peracid oxidation of aflatoxin B1 (AFB1) is reported. Using a modified chemical procedure, AFB1 was covalently bound to DNA, polyguanylic acid, or deoxyguanosine. Enzymatic binding was achieved by incubating AFB1 with rat liver postmitochondrial supernatants and DNA, together with the necessary cofactors. Enzymatic hydrolysis or acid hydrolysis of the DNA or polyguanosine to which AFB1 had been bound led to the release of AFB1 8,9-dihydrodiol, indicating that AFB1-8,9-oxide was formed by either enzymatic or chemical oxidation of AFB1. Two major products were formed by the peracid oxidation, and both were derived from guanine, probably through N-7 substitution. Covalent binding of AFB1 with guanine at the N-7 position is likely to induce frame-shift mutations due to intercalation of the AFB1 molecule together with base-substitution mutations as a result of misrepair. The results indicated that AFB1-8,9-oxide is formed during the peracid oxidation of AFB1 and that this metabolite reacts with nucleic acid. (14 Refs)

271. PREPARATION AND CHARACTERIZATION OF AFLATOXIN B1-L-(O-CARBOXY-METHYL) OXIME.

Chu FS, Hsia S, Sun PS
Univ. Wisconsin, Food Res. Inst., Madison, WI 53706
J Assoc Off Anal Chem; 60(4):791-794 1977

A method for preparation of aflatoxin B1-l-(O-carboxymethyl) oxime (afla B1 oxime) is described. The purpose of this preparation was to alter the aflatoxin in such a way as to produce a free carboxyl group which can then enter into a radioimmunoassay coupling reaction. Carboxymethylamine-HCl (79 mg) and aflatoxin B1 (52 mg) were refluxed for 2 hr in 40 ml of pyridine-methanol-water (1:4:1). The reaction mixture was incubated at room temperature overnight and concentrated by evaporation then redissolved in methanol-chloroform (1:9) and transferred to a Adsorbosil-5 chromatography column (2.2 x 50 cm). The column was eluted with chloroform and methanol-chloroform (1:9) and the eluate was monitored by thin layer chromatography. The product was crystallized in methanol saturated with hexane. Mass spectroscopy revealed a molecular ion peak at mass/electron charge (m/e) 385 and a base peak at m/e 311. The infrared spectrum showed a broad O-H stretching band in the 2500-3700 cm^{-1} region partially overlapping the C-H stretching region. This is indicative of a carboxylic acid functional group. Nuclear magnetic resonance analysis of the oxime established that the only difference between it and the parent compound was in the cyclopentane ring system. Afla B1 oxime was not toxic to 8-day-old chick embryos at a concentration of 3.48 microg/egg. (13 Refs)

272. REDUCTION OF AFLATOXIN B1 WITH ZINC BOROHYDRIDE: AN EFFICIENT PREPARATION OF AFLATOXICOL.

Hsia MT, Chu FS
Food Res. Inst., Univ. Wisconsin, Madison, WI 53706
Experientia; 33(9):1132-1133 1977

A procedure is presented for the production of synthetic aflatoxicol by treatment of aflatoxin B1 with ethereal zinc borohydride under essentially neutral conditions. (11 Refs)

273. COMPARATIVE STUDY OF THE FLUORESCENT CHARACTERISTICS OF SOLUTIONS OF AFLATOXINS AND PALMOTOXINS IN CHLOROFORM.

Uwaifo AO, Emerole GO, Bassir O
Dept. Biochemistry, Univ. Ibadan, Ibadan, Nigeria
J Agric Food Chem; 25(5):1218-1220 1977

Fluorescent characteristics of aflatoxins B1, B2, G1, and B3 and palmotoxins B0 and G0 were similar. Their fluorescence emission maxima fell within the wavelength range of 410-430 nanometers (nm), and they were all excited at 365 nm. Fluorescence intensity was as follows: G2 greater than G1 greater than G0 greater than B3 greater than B0 greater than B2 greater than B1. (15 Refs)

VIII. REVIEWS AND GENERAL STUDIES ON MYCOTOXINS

274. MYCOTOXINS.

Pitt JI
CSIRO Div. Food Res., North Ryde, NSW, Australia
Food Technol Aust; 125(6):291-294 1973

In contrast to microbial toxins which traditionally are antigenic proteins, fungal toxins are nonantigenic polycyclic compounds. They have been shown to cause sickness and death in epidemic proportions in humans, turkeys, ducklings, and trout. The classic example of mycotoxicosis is alimentary toxic aleukia, which affected Russians living in the grain belt and was due to grain which had overwintered in the field and became moldy with species of Fusarium and Cladosporium. In other examples, a toxin of Aspergillus flavus which grew in improperly dried peanuts caused 'Turkey X' disease, while toxin from the same fungus growing on cottonseed caused hepatomas in trout. These and related toxins are called aflatoxins. Aflatoxins also can cause hepatomas when administered to rats. Other carcinogenic mycotoxins are islanditoxin and luteoskyrin, produced by Penicillium islandicum, a contaminant in moldy rice, and patulin, produced by Penicillium expansum, the cause of a common apple rot. Evidence is mounting that some human liver cancer, which has an exceptionally high incidence in parts of Africa and Asia, may be caused by mycotoxins in foodstuffs. Analysis of agricultural or food commodities for the presence of aflatoxins, whether by chemical, chromatographic, or toxicological procedures, is difficult due to interfering compounds, sampling problems, and a lowness of the assays.

275. MYCOTOXINS

Wogan GN
Dept. Nutrition and Food Science, Massachusetts Inst.
Technology, Cambridge, Mass. 02139
Annu Rev Pharmacol; 15:437-451 1975

The current status, public health relevance, and illustrative examples of the mycotoxins problem are reviewed. The aflatoxins , the best characterized of the mycotoxins, chemically are highly substituted coumarins containing a fused dihydrofurofuran configuration; two series, the aflatoxin B, and G , derivatives are recognized. The aflatoxins are produced by a few strains of Aspergillus flavus or A parasiticus; sterigmatocystins are metabolites of A versicolor, A flavus, A nidulans, A rugulosum, A. Bipolaris species, and a penicillium. Despite wide variations in species responsiveness to acute toxicity and carcinogenicity, the liver is the main target organ for aflatoxin B1. Biochemical changes follow a consistent pattern of DNA and RNA polymerase inhibition. Feces represent the principal excretory route, and radioactivity is retained mainly in the liver. Metabolic transformations of aflatoxin B1 and G1 include ring hydroxylations, O-demethylation, epoxidation, and double bond hydration; all are demonstrated semiquantitatively in crude and purified microsomal preparations. Trichothecene mycotoxins (closely related sesquiterpenes) are produced by many species of Fusarium, Mycothecium, Trichothecium, Trichoderma, and Cephalosporium. A broad spectrum of biological responses is described, with the potency varying considerably with structural modification. Clinical and pathological responses noted include lesions of the oral cavity, gastrointestinal inflammation and hemorrhage, and suppression of rapidly proliferating tissues. Current trends in mycotoxin research include the development and application of new analytical methods, the isolation of fungi from foods, and the identification of the toxic agents produced. Assessment of the public health significance lacks quantitative cause-effect relationships, but suggests several circumstantial associations of mycotoxins with human disease.

276. INTERRELATIONSHIPS OF MYCOTOXINS WITH NUTRITION.

Hamilton PB
Dept. Poultry Science, North Carolina State Univ.,
Raleigh, NC 27607
Fed Proc; 36(6):1899-1902 1977

The interactions of mycotoxins with lipid, protein, carbohydrate, and vitamin nutrition are reviewed, with emphasis on aflatoxin and carcinogenicity. High-lipid diets have a mortality-sparing effect against aflatoxicosis; diets high in unsaturated fats have a growth-sparing effect. (40 Refs)

277. MYCOTOXINS AS CAUSE OF DISEASES IN MAN.

Gedek B
Fachbereich Tiermedizin, Universitat Munich,
Veterinarstrasse 13, 8 Munich 22, West Germany
Fortschr Med; 94(12):694-701 1976

A review of the sources, distribution, pathology, dose-response relations, environmental exposure, nutritional risk, metabolic fate, and carcinogenicity of mycotoxins is presented. The unusual sensitivity of the liver, regardless of portal of entry, is surveyed in depth. Cutaneous application of aflatoxin B1 , the most potent mycotoxin, and related compounds elicits parenchymal changes within 24 hr, with no residual reaction on the skin. No correlation can be established between the LD50 for a given species and the carcinogenic dose, nor between LD50 and hepatotoxic dose. There is no known structure common to tumorigenic mycotoxins, although a lactone ring, even if unsaturated in the a- and b-positions, occurs frequently. It would appear that mycotoxins function as alkylating agents, actively intervening in DNA-RNA synthesis. Mention is also made of the correlation between the extent of environmental contact with the fungus and neoplastic frequency. Cancer incidence is higher in homes or villages known to sequester the fungus. The mycoflora of a house, for example, has been matched to that of its leukemic residents.

278. AFLATOXIN.

Moyle, Newens, Strang
No affiliation given
Environ Health; 85(6):143 1977

Although a general correlation has been noted between contamination of food by aflatoxin and the incidence of liver cancers in humans, it has not been proven to be the causative agent. Maximum permitted levels of aflatoxin in animal feed are listed. (no Refs)

279. CURRENT ASPECTS OF THE AFLATOXIN PROBLEM.

O'Brien CA
College of Agricultural Sciences, Texas Tech University,
P.O. Box 4169, Lubbock, Texas 79409.
Southwest Vet; 29(3):226 1976

Aflatoxicosis can be described as a noninfectious disease caused by toxic metabolites of a common fungus, Aspergillus flavus, found worldwide in agricultural commodities and soils. Following mold contamination of food and feedstuffs, toxin may be detected within 3 to 7 days under favorable growth conditions of high temperature and humidity. Contamination normally occurs in storage following improper methods of harvesting, drying and handling. Aflatoxin poisoning is rare in practical livestock production, but outbreaks may appear during periods of unfavorable weather when cereals, oil seed crops and other commodities are stored in a high moisture state, or if the crops are damaged in the field by rain, snow, dew or by contact with wet ground. Aflatoxin is a cell toxin which is the most potent liver cancer producing agent known. It inhibits cell division and protein synthesis, and impairs growth, feed utilization, and reproduction. Depression of resistance to disease by low levels of ingested toxin is presently a matter of especial concern. (Author Abstract)

280. NATURALLY OCCURRING CARCINOGENS (MEETING ABSTRACT).

Wogan GN
Massachusetts Institute of Technology, Cambridge,
Massachusetts, USA
Third International Symposium On Detection And
Prevention Of Cancer; 6-7 1976

Many compounds of natural origin have been shown in bioassays to possess carcinogenic activity for animals, and in a few instances evidence of carcinogenicity has come from epidemiologic studies in human populations. Most of the known carcinogens are normal constituents of plants or plant products used as foods, herbal medicines or teas. Others are not normally present, but become contaminants of foods or beverages accidentally as in the case of mycotoxins produced by spoilage fungi. In many instances, carcinogenic principles have been chemically identified, but other natural products contain unidentified carcinogens, such as bracken fern, betel, tobacco, certain alcoholic beverages, and certain fungi. The term 'naturally occurring' has not been used to include substances (eg, polycyclic aromatic hydrocarbons) formed from natural products under extreme conditions such as pyrolysis. Among the best documented natural carcinogens are certain mycotoxins, including aflatoxins, sterigmatocystin, luteoskyrin and cyclochlorotin. Carcinogenic plant components that have been extensively studied include cycasin, some pyrrolizidine alkaloids, safrole, and certain antithyroid substances. Direct evidence of carcinogenic activity in man based on knowledge of human responsiveness is lacking in all cases. Epidemiologic evidence linking exposure to cancer incidence is suggestive of an etiologic role for a few of these agents (eg, aflatoxins), but for the most part assessment of carcinogenic risk to man must be based on estimates of human exposure and evidence of carcinogenicity in animals. (Author Abstract)

281. NATURALLY OCCURRING MUTAGENS OF BIOLOGICAL ORIGIN. A REVIEW.

Tazima Y
Natl. Inst. Genet., Mishima, Japan
Mutat Res; 26(4):225-234 1974

Naturally occurring mutagens were reviewed. Pyrrolizidine and some other alkaloids contained in higher plants (Senecio, Crotalaria, Heliotropium), cycasin in cycads, bracken toxins in ferns and several kinds of mycotoxins produced by fungi were covered. Chemical structures and major biological effects of these substances are discussed in relation to their carcinogenicity and mutagenicity. Cycasin and sinigrin are not mutagenic by themselves, but their enzymatic metabolites are known to be mutagenic. From the point of view of genetic hygiene the most important group may be the mycotoxins because these are contaminants of several sources of human food stuffs. One group of these substances, the aflatoxins, are the most active liver carcinogens and were discovered as the result of outbreaks of toxicosis in poultry, cattle, pigs, and sheep due to eating ground nuts. Another group of mycotoxins is the antibiotics, patulin and penicillic acid. The carcinogenic property of both substances was revealed following repeated sc injection in the rat. No evident carcinogenicity, however, has been observed yet in animals following ingestion. Mutagenic investigations of the mycotoxins are as yet very limited, but increasing evidence for a close relationship between carcinogenicity and mutagenicity suggests that most of them are mutagenic. In view of the probable significance of mycotoxins on the incidence of spontaneous mutations as well as etiology of cancer, the necessity for further investigation is stressed.

282. CANCER AND CARCINOGENESIS. (PP. 357-404)

Ball CR, Clayson DB
The Cell in Medical Science. Beck F, Lloyd JB, ed. New York, Academic Press, Vol. 4, 1976.

Cancer is a disease of multicellular organisms. It is believed to be the clinical manifestation of usually irreversible failure in the biological and biochemical mechanisms that maintain the integrity and relative size of tissue in the organism. Tumors can be induced easily in experimental animals, but it is difficult to develop methods to study the mechanisms of carcinogenesis. Slight variations in the experimental conditions may have an effect on tumor yield. Evidence that chemicals, viruses, and radiation can modify genetic material is summarized. In a unifying theory of carcinogenesis, the initial event can be regarded as a somatic cell mutation. This and other theories are discussed. Chemical carcinogens are not always industrial or synthetic substances; some of the most potent agents, such as the mold metabolite aflatoxin, are of natural origin. The great diversity in the chemical structure of carcinogens suggests a multiplicity of mechanisms for the chemical induction of cancer. Radiation induces damage in biological material as a result of the energy transferred to that material in decelerating a high-energy particle or in absorbing the electromagnetic radiations. The energy transferred is sufficiently high to cause the rupture of chemical bonds. Viruses act by imparting new information in the genome. The nature of the critical changes in the genome is still a matter of speculation. In cancer, the homeostatic mechanisms that maintain the integrity of the normal organism are lost. (90 Refs)

283. CARCINOGENS IN FOODS AND FOOD PRODUCTS. (PP. 67-73)

Sugimura T
Natl. Cancer Center Research Inst., Tokyo, Japan
Proceedings of the Eleventh Canadian Cancer Research Conference. Natl Cancer Inst Canada Toronto Ontario 6-8 May 1976, 1976.

Human beings are exposed to many carcinogens in foods. They may occur naturally in food sources, i.e., cycasin in cycad nuts and the active carcinogenic principle in bracken fern (a Japanese delicacy), or they may be naturally produced substances such as mycotoxins, which can contaminate food sources. Artificially introduced food additives may also be carcinogenic, such as the food preservative 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, or they may interact with naturally occurring substances to produce carcinogens, as when nitrite reacts with secondary amines to yield nitrosamines. Many substances in food may influence the formation of these nitrosamines: gallic acid (related to tannin) and 4-methylcatechol (related to chlorogenic acid in coffee beans) enhance, whereas ascorbic acid, ubiquitous in fruits, depresses nitrosamine formation. The carcinogenicity of fatty acid peroxides in food has also been claimed; antioxidants, such as a-tocopherol, may prevent this formation. Nutritional status may also influence carcinogenesis in humans: e.g., vitamin B2 deficiency and hepatic injury resulting from inadequate food intake may promote hepatocarcinogenesis, and large quantities of salt, which by itself can produce stomach ulcers in experimental animals, may enhance the action of gastrocarcinogens such as N-methyl-N'-nitro-N-nitrosoguanidine.

284. IS THE AFLATOXIN LEVEL IN PEANUT BUTTER HAZARDOUS TO MAN?

Gilchrist A, Fletcher DC
AMA Dept. Foods and Nutrition, Chicago, IL
JAMA; 238(6):518 1977

Although aflatoxins appear to be one of the most potent experimental hepatocarcinogens known, there is no direct evidence that they cause cancer in man. With current technology, they cannot be eliminated from foods. The geographic distribution of liver cancer appears to bear no relationship to the distribution of aflatoxin in food materials in the US. (3 Refs)

285. CARCINOGENS IN PLANTS AND MICROORGANISMS. (PP. 626-689)

Schoental R
Chemical Carcinogens. Searle CE, ed. Washington DC, American Chemical Society, ACS Monograph 173, 1976.

A literature review of naturally-occurring carcinogens in plants and microorganisms is presented. Pyrrolizidine alkaloids, constituents of essential plant oils, including methylenedioxy-, polymethoxyallyl-, and propenylbenzenes and related compounds, and "natural" estrogens, including the effects of oral contraception, were discussed in terms of chemical structure and versatility of unrelated metabolic pathways, both of which bear responsibility for tumorigenicity. Secondary metabolites of microorganisms, including aflatoxin and sterigmatocystin and other bifuranoid compounds, were also examined. The effect of griseofulvin, a metabolite of the mutant Penicillium griseofulvum, in arresting mitotic division in Walker carcinoma and lymphosarcoma was mentioned. The therapeutic potential of daunomycin, a secondary metabolite of Streptomyces peucetius, in leukemia was cited. Streptozotocin, a 2-deoxy-D-glucose derivative of N-methyl-N-nitrosourea and a secondary metabolite of Streptomyces achromogenes var 128, was noted for its therapeutic use in metastatic carcinoid and insulinomas, since it preferentially injures islet beta-cells. The tumorigenicity associated with the great variety of carcinogenic chemical structures can be subsumed under at least two factors: the presence of a reactive carbonyl group and an ethylene double bond in close proximity. During metabolic oxidation (epoxidation) the double bond is involved in transformation into polyfunctional intermediates which can interact with several reactive groups in the chromatin during momentary M-phase exposure. The carcinogenic residues form a stable "bridge" between the chromatin constituents, possibly inducing a permanent change in some chromosomes which leads to neoplasia. (380 refs)

286. THE CONTRIBUTION OF EPIDEMIOLOGY TO KNOWLEDGE OF CANCER.

Doll R
Dept. Regius Professor Medicine, Radcliffe Infirmary, Oxford, Great Britain
Rev Epidemiol Med Soc Sante Publique; 24(2):107-121 1976

The 5 major contributions of epidemiology to the knowledge of cancer are reviewed. First, it has demonstrated that all cancers that are at all common anywhere show a large amount of variation, sometimes several hundredfold. No cancers are so uniformly common that they would affect as many as 1% by 75 yr of age, in the absence of other causes of death, in all countries. Studies of migrant groups and changes with time show that this variation is largely due to environmental factors and that most cancers are, in principle, preventible. Secondly, the incidence of specific cancers can be correlated with the prevalence of suspicious factors in the environment. Recent correlations include those between esophageal cancer in Iran and the consumption of certain locally produced foods, between colon cancer and certain anaerobic bacteria in the feces, and between **liver cancer and the amount of aflatoxin in the diet.** These correlations do not provide direct evidence of causation, but do suggest ideas for investigation. Thirdly, epidemiology has provided means for testing hypotheses by relating the occurrence of particular types of cancer to the personal characteristics of individuals, either by studying the past experience of people with and without cancer or by following up people whose exposure to suspected agents has been previously defined. As a result, dozens of occupational hazards have been detected in industry, many more than have been detected by laboratory experiment. Fourthly, epidemiology has confirmed that an agent is a cause of cancer by monitoring the effects of intervention to reduce or prevent exposure. An example is provided by observation in British doctors, who have reduced their consumption of cigarettes to half the national average and have experienced a corresponding reduction in the risk of death from lung cancer. Finally, epidemiology has provided evidence of the quantitative and temporal relationships between exposure to carcinogenic agents and the development of cancer, which helps to elucidate the causative mechanisms of human cancer. (11 refs)

287. A BRIEF OVERVIEW OF THE TOXICOLOGICAL AND EPIDEMIOLOGICAL BACKGROUND TO THE DETECTION AND PREVENTION OF CANCER IN AGRICULTURAL WORKERS.

Kay K
Environmental Sciences Lab., Mount Sinai Sch. Medicine, City Univ. New York, New York, NY
Cancer Detect Prev; 1(1):107-128 1976

The epidemiologic and toxicologic data on the prevention and detection of cancer in agricultural workers is reviewed. The carcinogenic etiologic agents in agriculture (chlorinated hydrocarbons, thiourea compounds and carbamates, organophosphorus compounds, arsenic, other inorganic compounds, miscellaneous pesticides, fungicides and herbicides, and carriers and vehicles) are discussed. **Handlers of feed contaminated with carcinogenic mycotoxins may face a significant cancer risk** by inhalation and subsequent swallowing of mycotoxin-laden particles swept from the lungs and bronchi. In a study of feeds in the United States in 1974, mycotoxin contamination was suspected in 5.7% of 749 samples. It is concluded that, since agricultural workers receive the high exposures, research on prevention and detection of cancer among the workers is primary to assessing the overall public cancer hazard of mycotoxins and pesticides. (103 refs)

288. PRENATAL EXPOSURE AND CARCINOGENESIS.

Munoz N
International Agency Res. Cancer, Lyon, France
Tumori; 62(2):157-162 1976

The relationship between prenatal exposure to carcinogenesis and the risk of subsequent development of cancer is discussed. At least 29 chemicals induce tumors transplacentally in various experimental animals, including benzopyrene, methylcholanthrene, urethane, nitrosamines and **aflatoxin B.** The critical factor is the timing of the administration during pregnancy; the compounds act as transplacental carcinogens only when administered during the second half of pregnancy. They have an embryotoxic effect if administered early in pregnancy and a teratogenic effect if administered later in the first half of pregnancy. The studies on the risk of children developing leukemia, lymphoma, Wilm's tumor and other cancers after prenatal exposure to diagnostic radiation, stilbestrol and viral infections are reviewed. Experimental evidence suggests that the increased risk for cancer associated with prenatal exposure to a carcinogen persists for at least two generations. (22 refs)

289. TRANSPLACENTAL AND NEONATAL CARCINOGENESIS: EXPERIMENTAL APPROACH.

Vesselinovitch SD
Pritzker Sch. Med., Chicago, Ill.
Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April: 4-6,1973:135-150 1974

The susceptibility of the new-born mammal to chemical carcinogenesis during the prenatal period is supported by a review of experimental evidence from both animal and human sources in which either the mother or the animal offspring were exposed to carcinogens. Clinical evidence is presented from human transplacental carcinogenesis resulting from maternal exposure to diethylstilbestrol with consequent induction of vaginal cancer in female offspring. The effect of gestational age in maternal mice (at the time of carcinogen exposure) on the type of tumor produced and the effect of administering the procarcinogen, ethyl urea and its activator, sodium nitrite is described. The administration of ethylnitrosourea to newborn mice and the type of tumor produced correlated with the weanling's age at the time of carcinogen exposure. **Neonatal carcinogenicity of other substances such as aflatoxin B1,** benzidine and diethyl nitrosamine, which requires liver activation, are reviewed. Enhanced sensitivity to carcinogens of the fetus and newborn organism, demonstrated in animal studies suggests that the maximum nonhazardous level of carcinogen to a human mother may prove carcinogenic to her fetus.

APPENDIX:

Citations of Reviews Including Mycotoxin Carcinogenesis

290. SPECIAL REPORT: CHEMICAL CARCINOGENESIS.
Ryser HJ
Boston University School of Medicine, Boston MA
CA; 24(6):351-360 1974

291. HEPATOCARCINOGENESIS BY CHEMICALS.
Miller EC, Miller JA
McArdle Lab. Cancer Res., Univ. Wisconsin Medical Center, Madison, WI
Prog Liver Dis; 5:699-711 1976

292. CHEMICAL CARCINOGENS.
Van Wassenhove F, De Kimpek N, Schamp N
Laboratorium voor Organische Scheikunde, Fakulteit van de Landbouwwetenschappen, R.U.G., Ghent, Belgium
Ingenieur; 46(5):121-130 1977

293. THE CHEMICAL INDUCTION OF CANCER. (PP. 163-179)
Clayson DB
Biology of Cancer. Ambrose EJ, Roe FJ, ed. New York, Halsted Press, 2nd ed., 1975.

294. THE CARCINOGENIC ACTION OF DRUGS.
Clayson DB, Shubik P
Eppley Inst. Cancer Res., Univ. Nebraska Medical Center, Omaha, NB
Cancer Detect Prev; 1(1):43-77 1976

295. ACTIVATION AND INACTIVATION OF CHEMICAL CARCINOGENS AND MUTAGENS IN THE MAMMAL.
Magee PN
Middlesex Hosp. Med. Sch., London, England
Essays Biochem; 10:105-136 1974

296. STRATEGY FOR DETECTION OF CANCER HAZARDS TO MAN.
Doll R
Dept. Medicine, Univ. Oxford, Oxford, England
Nature (Lond); 265(5595):589-596 1977

297. AETIOLOGICAL FACTORS IN HUMAN CANCER.
Higginson J, MacLennan R, Muir CS
The Physiopathology of Cancer. Homberger F, Brennan MJ, Krakoff IH, ed. Basel, S Karger, vol. 2, 1976.

298. PREDICTIVE ONCOLOGY: FOLLOWING PATIENTS AT HIGH CA RISK.
Anonymous
No affiliation given
Patient Care; 10(6):56-70 1976

299. EPIDEMIOLOGY OF SOME HUMAN CANCERS: (3) LIVER. (PP. 224-232)
Hutt MS, Anthony PP
Scientific Foundations of Oncology. Symington T, Carter RL, ed. London, William Heinemann Medical Books Ltd, 1976.

300. ENVIRONMENTAL RISKS RELATED TO CANCER. (PP. 85-92)
Peacock PB
Cancer, The Behavioral Dimensions. Cullen JW, Fox BH, Isom RN, ed. New York, Raven Press, 1976.

301. CARCINOGENIC FACTORS IN THE ENVIRONMENT.
Schmahl D
German Cancer Res. Ctr., Heidelberg
Verh Dtsch Ges Inn Med; 78:25-34 1972

302. ESTIMATION OF RISKS DUE TO ENVIRONMENTAL CARCINOGENESIS.
Cranmer MF
National Center for Toxicological Research, Jefferson, Ar. 72079
Proc Am Assoc Cancer Res; 16:208 1975

303. CHEMICAL CARCINOGENS IN THE HUMAN ENVIRONMENT: PROBLEMS AND QUANTITATIVE ASPECTS.
Preussmann R
Deutsches Krebsforschungszentrum, Institut fur Toxikologie und Chemotherapie, Im Neuenheimer Feld 280, D-6900 Heidelberg, West Germany
Oncology; 33(2):51-57 1976

304. CHEMICAL CARCINOGENS IN THE HUMAN ENVIRONMENT.
Preussmann R
Deutsches Krebsforschungszentrum, Heidelberg, West Germany
Handb Allg Pathol; 6:421-594 1975

305. ENVIRONMENTAL MUTAGENS.
Bendix S
Bendix Res., 1103 The Alameda Berkeley, Calif. 94707
Science; 184(4133):138-189 1974

306. DIET. (PP. 201-224)
Berg JW
Persons at High Risk of Cancer An Approach to Cancer Etiology and Control; Edited by J. F. Fraumeni, Jr. (New York: Academic Press, Inc.): 1975

307. NUTRITION AND CANCER: INGESTION OF FOODBORNE CARCINOGENS.
Higginson J, Terracini B, Agthe C
Int. Agency for Res. on Cancer 150 Cours Albert Thomas, 69 Lyon 6cme France
Cancer Epidemiology and Prevention, Current Concepts. Edited by D Schottenfeld Springfield IL, Charles C Thomas, pp. 177-206, 1975.

308. RENAL CARCINOGENESIS.
Hamilton JM
Dept. Experimental Pathology and Cancer Res., Sch. Medicine, Univ. Leeds, Leeds, England
Adv Cancer Res; 22:1-56 1975

309. COLORECTAL CARCINOGENESIS.
Burdette WJ
U. Texas Med. Sch., Houston
Cancer Suppl; 34(3):872-877 1974

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